

(19)

Europäisches Patentamt

European Patent Office

Office européen des brevets



(11)

**EP 0 814 161 A1**

(12)

**EUROPEAN PATENT APPLICATION**

(43) Date of publication:

29.12.1997 Bulletin 1997/52

(51) Int. Cl.<sup>6</sup>: **C12N 15/29**, C12N 15/82,

C07K 14/415, A01H 5/00,

C12Q 1/68

(21) Application number: **96110088.0**

(22) Date of filing: **21.06.1996**

(84) Designated Contracting States:

**AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC  
NL PT SE**

Designated Extension States:

**LT LV SI**

• **Palme, Klaus, PD Dr.**

**50259 Pulheim (DE)**

• **Wismann, Ellen Dr.**

**6369 BD Simpelveld (NL)**

• **Saedler, Heinz, Prof. Dr.**

**50829 Köln (DE)**

(71) Applicant:

**Max-Planck-Gesellschaft zur Förderung  
der Wissenschaften e.V.  
80539 München (DE)**

(74) Representative:

**Maiwald, Walter, Dr. Dipl.-Chem.**

**Maiwald & Partner,**

**Pocistrasse 11**

**80336 München (DE)**

(72) Inventors:

• **Gälweiler, Leo**

**55595 St. Katharinen (DE)**

Remarks:

The applicant has subsequently filed a sequence listing and declared, that it includes no new matter.

(54) **Genetic control of polar auxin transport in plants and manipulation of plant growth, architecture and morphogenesis**

(57) This invention relates to the control of polar auxin transport or auxin activity and of plant hormone levels at the molecular level. It particularly relates to nucleotide sequences of the pin gene, their derivatives and fragments and the encoded gene products involved in polar auxin transport, and a method for the production of new plants carrying at least one of the nucleotide sequences of the invention. Further, the invention relates to new plants showing altered plant growth, architecture, morphogenesis, branching, flowering, fruit production and/or other developmental features affected by auxin.

**EP 0 814 161 A1**

## Description

This invention relates to the control of polar auxin transport and of plant hormone levels at the molecular level. It particularly relates to nucleotide sequences of the *pin* gene, their derivatives and fragments and the encoded gene products involved in polar auxin transport, and a method for the production of new plants carrying at least one of the nucleotide sequences of the invention. Further, the invention relates to new plants showing altered plant growth, architecture, morphogenesis, branching, flowering, fruit production and/or other developmental features affected by auxin.

Plant hormones are naturally occurring substances, effective in very small amounts, that act as signals to stimulate or inhibit growth or regulate some other developmental process in higher plants. One of the most common plant hormones is indole-3-yiacetic acid (IAA), which is often referred to as "auxin" and which was the first plant hormone to be identified. The auxin biosynthetic pathway is not known with certainty, but there appears to be one major pathway for the synthesis of IAA in plants via tryptophan, and another one via indole. IAA is found in plants in free form or in the form of ester or amide conjugates.

The definition of a hormone includes the stipulation that the hormone moves through the organism from its site of synthesis to its site of action. Mainly, auxin is synthesized in meristematic and embryonic tissues and juvenile parts of the plant (e.g. young leaves). In dicotyledones the apex, i.e. the tip of the main shoot, is the most important IAA source. Auxin is transported down the stem by a specific mechanism known as the auxin transport system. This energy-dependent polar basipetal (i.e. towards the root) transport, being gravity independent, occurs mainly in vascular tissues. The transport velocity is generally 5-15 mm/h.

Auxin regulates many aspects of plant growth and development. In addition to the regulation of cell elongation, auxin is involved in the control of several other developmental processes throughout the plant. Auxin is well established as the signaling molecule in phototropism. It regulates the elongation of young internodes and the enlargement of the young leaf blades. It also initiates cell differentiation in both leaves and internodes as it stimulates their enlargement or elongation. While promoting the growth of these tissues and organs, it inhibits the development of lateral buds. This leads to a condition known as apical dominance. Auxin inhibits the formation of the abscission zone at the base of the leaves, fruits, and other organs that allows them to drop from the plant. Auxin also regulates vascular tissue differentiation and cell division in the vascular cambium, which of course is involved in secondary growth, and it has been implicated in the maintenance of tissue polarity.

Although the molecular mechanisms of polar auxin transport are presently not fully understood in detail, it seems likely that specific transport proteins, so-called auxin-efflux-carrier proteins, are involved. Results obtained using chemical compounds which inhibit polar auxin transport, like the phytotropin N-(naphth-1-yl)phthalamic acid (NPA) or 2,3,5-triiodobenzoic acid (TIBA), indicate that such inhibitors compete with IAA for specific auxin receptor proteins, which interact with the carrier proteins, and/or more directly for the specific auxin-carrier proteins (Jacobs and Rubery (1988), Science 241, 346-349; Rubery (1990), Soc. Exp. Biol. Symp. 44, 119-146).

The manipulation of polar auxin transport and the developmental processes affected by auxin is a very important aim of molecular plant breeding, and some efforts have been made to isolate and characterize the genes involved. Several studies focused on plant mutants known to be defective in active auxin transport. For instance, the *pin1-1* mutant of *Arabidopsis thaliana* having a "pin" shape morphology shows several structural abnormalities in different organs at different stages of plant growth. In some cases this mutant forms flowers with abnormal structures at the top of inflorescence axes; in other cases, no floral buds are formed on the axes (Okada et al. (1991), The Plant Cell 3, 677-684). The polar transport activity in the *pin1-1* mutant and in the *pin1-2* mutant, an independently isolated allelic mutant which shows similar phenotypes as *pin1-1*, was decreased to 14% and 7% of wild type, respectively (Okada et al., *supra*). This observation, together with the finding that the mutant phenotypes are exactly the same in wild-type plants cultured in the presence of the auxin polar transport inhibitor NPA, led to the conclusion that the primary function of the *pin1* gene is auxin polar transport. Using multiply marked tester strains the *pin* locus was mapped and found to be 3.0 +/- 0.4 map units below *crabs claw* which in turn is approximately 0.2 map units below *Apetala* on chromosome 1 of *Arabidopsis thaliana* (Bennett et al. (1995), The Plant J. 8, 505-520; Okada and Shimura in: Bowman J. (ed.) (1994), *Arabidopsis*, an Atlas of Morphology and Development, Springer Verlag Berlin, New York). However, although several attempts were made over the last years in order to isolate the *pin1* gene by chromosome walking or other cloning strategies, none was successful.

Other approaches for modifying hormone controlled processes in plants have been reported. US-A-4401454, for example, discloses effects on the regulation of plant growth, including increased yields, enhanced auxin activity, inhibition of terminal growth, etc., by application of phosphonic acid derivatives. Similarly, EP-A-215445 relates to new pyrazoloisoquinoline derivatives which have auxin transport inhibiting activity and can be applied to plants as plant growth regulators to control plant height or to control abscission. WO 96/09755 describes a method for obtaining plants with delayed or inhibited fruit ripening and/or vegetable tissue senescence comprising selection and breeding of plants to produce strains with low polar auxin transport and low inhibition of cytokinin conjugation.

Also, genetic approaches for the manipulation of developmental processes in plants have been reported. WO 94/10831 discloses the transformation of plants with a DNA sequence encoding a group 3 late embryogenesis abun-

dant (LEA) protein and the production of shorter, stronger and more branched plants. WO 92/02622 relates to plants transformed with a gene complex from melon and resulting delay in fruit ripening. EP-A-445658 describes the isolation of a IAA synthetase gene from *Enterobacter cloacae* which could be introduced into microorganisms or plants. WO 96/00291 discloses the expression of UDP-glucose indol-3-ylacetyl-glucosyl transferase (IAGlu Transferase) DNA in transgenic plants. By use of these non-naturally occurring nucleotide sequences, the gene product thereof being involved in the conjugation of IAA with carbohydrates, control of IAA levels and plant growth is said to become possible.

However, so far no gene or gene product directly involved in, or even essential to, polar auxin transport has been characterized. Since the *pin* gene product has a direct influence on, inter alia, structure, development, initiation and differentiation of the vascular system (and thereby is likely to affect sink-source relationships), plant growth, flowering, fruit ripening, abscission and cambial differentiation, the nucleotide sequences of the invention can be used to manipulate one or more of these features in transgenic plants.

The *pin1* gene has now, however, surprisingly been successfully cloned, characterized and sequenced, as described hereinafter. Detailed descriptions of the transposon tagging strategy used, the *pin1* gene and its gene product are given below. Further, the *pin2* gene from *A. thaliana* has also been cloned using the same transposon tagging strategy. The nucleotide sequence of the *pin2* gene is also given below. These nucleotide sequences now provide the possibility to manipulate several different processes of growth and development in higher plants which are controlled by auxin directly or indirectly.

Thus, one object of the invention is to provide a tool for modifying and controlling the synthesis of plant fibres. Since the *pin* gene product controls initiation and differentiation of the vascular system, fibre synthesis in plants is expected to be directly influenced by modifying *pin* gene expression. Plant fibres, being a renewable resource, are used in the production of pulp, paper, textiles and the like. Further, plant fibres are used in fibre reinforced composites, construction and building materials, (biodegradable) geotextiles, nonwovens, insulation materials and asbestos replacing materials. Fibres can be further used in mixture yarns with cotton, silk or synthetics. Cellulose from fibre crops may be successfully applied as raw material for viscose production. By manipulation of *pin* gene expression in plants it becomes possible to improve the control of yield and quality of plant fibres.

Another object of the invention is to improve wood quality and wood yield by regulation of wood formation. Since cambial growth is controlled by auxin through morphogenetic regulation of vascular tissues, wood production is expected to be efficiently influenced by manipulating polar auxin transport in plants. Thus, as a result of altered *pin* gene expression in transgenic plants, cambial division and therefore wood formation can be inhibited or enhanced, e.g. in woody dicotyledons and gymnosperms.

Another object of the invention is to engineer novel plant architectural traits in plants. The ability to manipulate branching has numerous applications in both agriculture and horticulture. For example, reduced branching in apple trees would lead to improvements in mechanical harvesting of fruit, whereas improved root colonisation in commercial tree plantations would have benefits for both soil and tree stability. While the pattern of lateral root initiation is further central to the efficient exploitation of water and nutrient supplies, the degree of shoot branching affects important factors such as light harvesting and fecundity. The ability to control branch development and to modify branching pattern is therefore a powerful tool for plant breeders. Further, reduced branch development would lead to improvements in pulp-ing efficiency. The regulation of plant architecture, e.g. in poplar trees, would ensure a more reproducible product for the construction industry. Producing synchronous branching, which would result in a reduction of yield loss, is also desired by plant breeders, e.g. in oilseed rape.

Other objects of the invention are based on further characteristics of plants which are influenced directly or indirectly by polar auxin transport and auxin levels. For instance, one object is to enhance parthenogenetic fruit production, e.g. in tomato and cucumber. Since parthenogenesis is stimulated by IAA and also some synthetic auxins, enhanced expression of the nucleic acid sequences of the invention, being either systemic, tissue-specific, developmental specific and/or inducible by biotic or abiotic stimuli, could satisfy this need in plant breeding.

Further, there is a need to make harvesting or picking of fruits, for example citrus and malaceous fruits, easier and more efficient. Since the force with which a plant holds on to its fruit, is also affected by auxin levels, this problem could also be solved by manipulation of polar auxin transport in plants due to accordingly tailor-made expression of the DNA sequences of the invention in transgenic plants.

The influence of IAA and auxins in general on root formation has already been mentioned. Therefore, the object of stimulating root formation, e.g. in cuttings, can also be attained by the manipulation of auxin transport and auxin levels by use of the DNA sequences of the invention.

Another object in agriculture and horticulture is to influence fruit ripening, fruit colour and/or senescence. These traits can also be enhanced, i.e. accelerated and/or increased, inhibited or delayed in transgenic plants which have altered polar auxin transport due to expression (e.g. overexpression, antisense-expression or controlled expression) of the DNA sequences of the invention.

Another object in agriculture is to control abscission and dropping of fruits. Depending on the kind of plantation and plant, dropping of fruits prior to harvest has sometimes to be prevented, e.g. in citrus fruits and malaceous fruits, whereas sometimes abscission is desired, e.g. in cotton plants ready for harvest. Shedding of leaves and fruits can also

be controlled, i.e. for instance stimulated or delayed, by specific expression of the DNA sequences of the invention in transgenic plants.

Until now, plant breeders influence, stimulate or inhibit some of the above-mentioned traits by local or systemic application of auxins or polar auxin transport inhibitors to plants. However, this application is time- and energy-consuming, labour-intensive and often also cost-intensive.

It is, therefore, an object of this invention to provide a tool for the manipulation of the above mentioned traits in plants by control of polar auxin transport, in particular, to provide nucleotide sequences encoding the pin gene product and DNA molecules containing these sequences and to provide the proteins or protein fragments encoded by them. Further objects of this invention are the provision of nucleic acid molecules comprising the coding region of the pin1 gene or pin2 gene or fragments thereof, the provision of vectors and microorganisms, the uses of which open the possibility to produce new plants which have altered polar auxin transport activity and altered traits influenced by auxin transport and auxin levels, and the provision of a method for producing new plants which exhibit an altered polar auxin transport and altered auxin dependent traits in comparison to wild-type plants.

These and other objects are attained by the subject matters defined in the attached independent claims. Preferred embodiments are notable from the dependent claims and the present description.

The invention provides DNA sequences that code for proteins which are involved in polar auxin transport. Further the invention provides DNA sequences that code for proteins which are essential for polar auxin transport activity. Further the invention provides the pin gene product and predicted characteristics of this protein. As will be described below, our studies strongly support the idea that the pin protein is located to the plasma membrane and/or associated with the cytoskeleton and further is an auxin receptor protein and/or an auxin carrier protein and/or in contact with an auxin receptor protein and/or an auxin carrier protein. Without intending to be bound by any scientific theory or explanation, the invention thus also provides DNA sequences that code for proteins which control the activity of the auxin efflux carrier proteins or of other proteins involved in polar auxin transport.

In particular, the invention provides a full size cDNA clone (pcpin1/23) of the pin1 gene from Arabidopsis thaliana.

The invention further provides a full size cDNA clone (pcpin2/4) of the pin2 gene from Arabidopsis thaliana.

The cDNA clone of the pin1 gene was transformed in Escherichia coli and the E. coli strain containing the cDNA clone pcpin1/23 was deposited at the Deutschen Sammlung von Mikroorganismen (DSM), Mascheroder Weg 1B, D-38124 Braunschweig, Germany, in accordance with the requirements of the Budapest Treaty, on June 14, 1996 and received the deposit number DSM 10712.

Further, the invention provides preferably the protein encoded by the cDNA clone pcpin1/23 as well as the protein encoded by the cDNA clone pcpin2/4. These proteins are directly involved in polar auxin transport and appear to be auxin-specific receptors and/or auxin-specific efflux carrier proteins. The characteristics of the protein encoded by the pin1 gene are given below.

In the context of this invention the pin protein is referred to as the pin gene product, which is involved in polar auxin transport in plants. As mentioned above, it is known from studies on plant mutants that plants which are impaired in polar auxin transport have a "pin" shape and are often referred to as "pin"-formed mutants.

Further this invention provides DNA sequences and nucleic acid molecules that (i) hybridize to the DNA sequences of the invention or fragments thereof, (ii) are allelic derivatives of the DNA sequences of the invention or fragments thereof, and/or (iii) are DNA sequences which differ from the DNA sequences of the invention or fragments thereof as a result of the degeneracy of the genetic code.

In the context of this invention "active fragment" means in general that the fragment is sufficient for influencing polar auxin transport in plants. In detail, "active fragment" refers to fragments that are capable to provide altered plant traits through overexpression, antisense-expression or controlled expression of the fragments.

The nucleic acid molecules of the invention can be any form of nucleic acid, in particular DNA or RNA molecules, e.g. cDNA, genomic DNA, mRNA, etc. The nucleic acid molecules can be naturally occurring molecules or they can be produced by chemical processes or by methods of molecular biology known to a person skilled in the art.

The invention also provides nucleic acid molecules in which the DNA sequences of the invention are combined with regulatory elements that provide their transcription and, if desired, their translation in the plant cell. Provision of the DNA sequences and nucleic acid molecules of the invention opens the possibility to manipulate plant cells, plant parts or whole plants by genetic engineering to exhibit altered polar auxin transport activity or auxin activity.

Further, the invention provides nucleic acid molecules that provide tissue-specific, development specific, inducible and/or systemic expression of the DNA sequences of the invention. It is particularly advantageous that the DNA sequences of the invention can be expressed in a controlled fashion, i.e. dependent on the trait that is influenced by polar auxin transport and which is to be enhanced or reduced, i.e. the DNA sequences of the invention can be expressed in transgenic plants in a "tailor-made" fashion. In general, polar auxin transport or auxin activity can be increased, e.g. by overexpression or regulated expression of the DNA sequences of the invention, or it can be reduced by antisense-expression or other means that reduce homologous gene expression and/or polar auxin transport activity.

The invention further provides vectors and microorganisms which contain DNA sequences and nucleic acid molecules, respectively, that code for proteins directly involved in auxin transport or auxin activity. The invention thus also

provides vectors, in particular plasmids, cosmids, viruses, bacteriophages and other vectors used in molecular biology and genetic engineering, which contain the DNA sequences of the invention and which, if desired, can be used for transferring the DNA sequences of the invention to plants and plant cells. The invention also provides nucleic acid molecules and vectors, in which the DNA sequences of the invention are combined with regulatory sequences that provide transcription and translation in procaryotic and eucaryotic cells. If desired, the nucleic acid molecules and vectors may also contain additional enhancer sequences or other regulatory sequences. These regulatory sequences comprise also signal sequences, that provide the transport of the gene product to a specific cell compartment.

The invention also relates to microorganisms, such as bacteria, viruses, fungi, etc., which contain the DNA sequences of the invention.

The invention also provides new plants, which exhibit altered auxin transport and auxin activity and altered developmental and morphogenetic traits in comparison to wild-type plants. Some examples for traits which may be altered in plants through expression of the DNA sequences of the invention have been already mentioned. In particular, the invention provides plants which show altered plant architecture, e.g. reduced or enhanced branching, altered plant growth, e.g. reduced or enhanced height, altered fruit ripening, altered abscission, altered wood production, altered flowering and/or altered root formation.

The invention comprises transgenic plants, in the cells of which the DNA sequences of the invention are integrated in the plant genome. Further the invention comprises plants, in the cells of which the DNA sequences of the invention are located in a self-replicating system.

The plant that may be transformed with the DNA sequences or nucleic acid molecules of the invention can be any plant. Dependent on the trait desired to be altered, it may be a gymnosperm, dicotyledon or monocotyledon. Examples for gymnosperms are pine, spruce, fir, Douglas fir, Hemlock fir, larch, yew, cedar, juniper. Examples for monocotyledons are plants which belong to the genus of wheat (*Triticum*), barley (*Hordeum*), rye (*Secale*), oats (*Avena*), rice (*Oryza*), maize (*Zea*), millet (*Sorghum*), *Panicum*, *Pennisetum*, *Setaria*, sugar cane, bamboo. Examples for dicotyledons, and other plants, e.g. fibre plants, are cotton, leguminous plants, alfalfa, soybean, (oilseed) rape, tomato, potato, sugar beet, ornamentals, trees, e.g. poplar, sequoia (giant redwood), aspen, birch, eucalyptus, fruit plants and trees, e.g. apple, citrus, pineapple, pear, cherry, grape, banana, soft fruit plants, tea, coffee, cocoa plants, tobacco, sisal, hemp, coir, ramie, sun hemp, sansevieria, maur hemp, pita floja, flax, palms, silk cotton tree (kapok tree), coconut, jute, kenaf, miscanthus.

The invention also includes plant reproduction products of the plants of the invention, e.g. seeds, fruits, cuttings, tubers, rhizomes, and parts of the plants, as protoplasts, plant cells, calli or roots.

The invention further comprises host cells, in particular procaryotic and eucaryotic cells, which may be transformed or infected with a nucleic acid molecule or a vector of the invention and cells which are derived from those host cells and which contain the nucleic acid molecules or vectors of the invention. The host cells may be bacteria, fungi, plant cells and animal (mammalian) cells.

The invention provides methods for producing plants with altered auxin transport or auxin activity, and plant transformation methods which can be used to produce new plants with altered polar auxin transport and altered auxin transport or auxin dependent traits.

For the production of such new plants several different methods can be applied in accordance with this invention. On the one hand, plants or plant cells can be modified by common transformation methods known in genetic engineering to that extent that the new DNA sequences are integrated in the plant genome, i.e. stable transformants are created. On the other hand, the nucleic acid molecules of the invention, expression of which may result in altered auxin transport activity, can also be introduced in the plant or plant cell as self-replicating system. For instance, the nucleic acid molecules of the invention may be contained in a virus that infects the plant or plant cell.

The invention comprises a method for producing these new plants based on the DNA sequences of the invention and their transfer to plant cells and their expression in plants, which, if desired, may be controlled. By provision of the DNA sequences of the invention it is now possible to modify plant cells by genetic engineering so that they display altered auxin transport or auxin activity, in comparison to wild-type cells.

One method of the invention for the production of new plants which exhibit altered polar auxin transport or auxin activity as a result of expression of the DNA sequences of the invention, generally comprises the following steps:

- a) manufacture of an expression vector, comprising (i) a promoter region that provides transcription and translation, (ii) at least one DNA sequence that codes for the *pin* protein or an active fragment thereof, wherein this DNA sequence is combined in sense orientation to the 3' end of the promoter, and if desired or necessary (iii) a termination signal for transcription termination and addition of a poly-A-tail to the respective transcript, wherein the termination signal is combined to the 3' end of the coding region;
- b) transformation of plant cells with the expression vector produced in step a);
- c) regeneration of transgenic plants and, if desired, breeding of the plants.

In an alternative method according to this invention, the coding region of the *pin* gene or fragments thereof can be combined with an promoter in such a way that they are oriented in the antisense orientation.



In another alternative inventive method, the DNA sequences of the invention can be introduced in plant cells or plants as self-replicating system.

The invention comprises uses of the genetic and protein materials, especially including the use of the new DNA sequences and molecules for the production of plants which have an altered polar auxin transport or auxin activity. Further, the invention includes the use of the new DNA sequences and molecules for the production of plants in which plant growth, architecture, branching, wood production, morphogenesis, differentiation of the vascular tissues, abscission, flowering, fruit ripening, root formation and/or other auxin controlled developmental processes are altered.

The invention includes the use of the DNA sequences of the invention or the fragments thereof and the use of the proteins of the invention or the fragments thereof for the isolation of *pin* genes, fragments thereof or DNA sequences which are homologous to the DNA sequences of the invention and the isolation of *pin* proteins, fragments thereof or proteins which are homologous to the proteins of the invention. The invention also includes the use of the DNA sequences of the invention or fragments thereof and the use of the gene product or fragments thereof for the isolation of DNA sequences that code for proteins involved in polar auxin transport.

Thus the present invention incorporates any possible use of the DNA sequences of the invention, expression of which results in altered auxin transport or auxin activity, as well as the use of the proteins of the invention or fragments thereof, which have polar auxin transport activity.

In general, any regulatory sequences which fulfils the prerequisite of providing the desired gene expression pattern in the plant of choice can be used in order to express the DNA sequences of the invention in plants and to manipulate auxin transport dependent plant traits in a tailor-made fashion. These can be constitutive promoters, tissue-specific promoters, e.g. such which provide stem-specific expression, developmental specific promoters or promoters which are induced by abiotic or biotic stimuli. One example for a constitutive promoter that can be used in the scope of the invention is the 35S RNA promoter from Cauliflower Mosaic Virus (described e.g. in Rogers *et al.* (1987) *Meth. Enzymol.* **153**, 253-277). Other constitutive promoters that are active in plants are known to the person skilled in the art. Examples for tissue-specific promoters are, for instance, seed specific promoters (e.g. in Plant *et al.* (1994) *Plant Mol. Biol.* **25**, 1193-1205), vascular tissue specific promoters (e.g. in Guevera-Garcia *et al.* (1993) *The Plant J.* **4**, 495-505; Brears *et al.* (1991) *The Plant J.* **1**, 235-244), and stem specific promoters. Examples for developmental specific promoters or hormone induced promoters are e.g. given in Vögeli-Lange *et al.* (1994), *Plant Mol. Biol.* **25**, 299-311; Langridge *et al.* (1989) *Proc. Natl. Acad. Sci. USA* **86**, 3219-3223. Promoters which are induced by abiotic stimuli comprise those which are stimulated by chemicals, ozone, UV light, extreme temperatures, dryness, salt, etc., and which are described e.g. in Williams *et al.* (1992), *Biotechnology* **10**, 540; Schulze-Lefert *et al.* (1989) *EMBO J.* **8**, 651-656; Schulze-Lefert *et al.* (1989) *Plant Cell* **1**, 707-714. Promoters which are induced by biotic stimuli comprise promoters which are induced by pathogens, e.g. fungi, bacteria, viruses, insects and nematodes. Such promoters are known to the person skilled in the art and given in the literature, e.g. Martini *et al.* (1993) *Molecular and General Genetics* **236**, 179-186, or WO 94/17194.

In case useful promoters are not already known, the strategy and methods for the isolation of such promoters are known to the person skilled in molecular biology. In general, in a first step poly(A)<sup>+</sup> is isolated from a certain tissue, a plant or tissue of a certain developmental stage, or a plant or tissue treated with a certain stimuli, and a cDNA library is established using common means and methods of molecular biology. In parallel a second cDNA library is established from other tissues, plants or tissues of another developmental stage, or non-treated plants or tissues.

Then, in a second step, those clones are identified by differential hybridization, the corresponding poly(A)<sup>+</sup> of which are only expressed in the respective tissue, during the respective developmental stage or after treatment with an biotic or abiotic stimulus. Subsequently, the cDNA clones isolated by following the described strategy are used in order to isolate the corresponding promoters, which can then be used in order to express the DNA sequences of the invention in plants. By this way, the DNA sequences of the invention can be expressed in a controlled fashion in any tissue or developmental stage, or their expression can be induced by a certain treatment, whenever expression is desired in the whole plant or in certain plant parts.

When the DNA sequences of the invention are used in order to inhibit or reduce gene expression of homologous genes in transgenic plants, the DNA sequences provided by the invention can also be expressed in the antisense orientation. Antisense expression can also be controlled by constitutive, tissue-specific, developmental specific or otherwise inducible promoter sequences.

The invention also comprises nucleic acid molecules that code for proteins which have auxin transport activity or fragments thereof which hybridize to the DNA sequences and nucleic acid molecules of the invention. In the context of the invention, the term "hybridization" means a hybridization under conventional conditions, preferably under stringent conditions, as e.g. described in Sambrook *et al.* (1989), *Molecular Cloning: A Laboratory Manual*, 2nd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. Nucleic acid molecules that hybridize with the molecules of the invention may isolated e.g. from genomic or cDNA libraries. Identification and isolation of such nucleic molecules can be carried out using the nucleic acid molecules of the invention or fragments or reverse complements thereof, e.g. by hybridization under standard conditions (Sambrook *et al.*, *supra*).

Thus the invention also comprises the use of the DNA sequences of the invention or fragments thereof for the identification and isolation of homologous sequences from plants or other organisms.

For instance, nucleic acid molecules which display exactly or essentially the nucleic acid sequences of the invention or portions thereof can be used as hybridization probes. Also synthetic fragments, which are synthesized using common synthesis processes and which correspond basically to one of the DNA sequences or nucleic acid molecules of the invention can be used as hybridization probes. When genes are identified and isolated, which hybridize to the DNA sequences of the invention, it is necessary to determine their sequence and the sequence and characteristics of the proteins encoded by them. The man skilled in the art has a variety of biochemical, biotechnological and genetic engineering methods for the characterization of the nucleic acid molecules and the proteins at his disposal.

The molecules that hybridize to the nucleic acid molecules of the invention comprise also fragments and (degenerated or allelic) derivatives of the nucleic acid molecules described herein. The terms "derivatives" means in this context that the sequences of such molecules differ from the molecules of the invention (as herein described) in one or more positions and show a high degree of homology to the sequences provided by the invention. Homology means an identity in sequence of at least 40 %, in particular of at least 60 %, preferably of more than 80 % and more preferably of more than 90%.

The deviations from the sequences of the invention may be generated by deletion, addition, substitution, insertion or recombination.

Homology further means that there is a functional and/or structural equivalence between the respective nucleic acid molecules and the proteins encoded by them. Usually the nucleic acid molecules which are homologous to the molecules of the invention and which are derivatives of these molecules, are variations or modification which have the same biological function. These variations may be naturally occurring variations, e.g. related sequences from other organisms, or they can be mutations, which may be either spontaneous or artificially introduced (e.g. by targeted mutagenesis). Further, the variants can be synthetically produced sequences. Also the allelic variants may be naturally occurring or synthetically produced variants, or variants generated by recombinant DNA techniques.

Usually the proteins encoded by the different variants and derivatives of the nucleic acid molecules of the invention have common characteristics. Such characteristics are e.g. the molecular weight, enzyme activity, efflux activity, binding capacity, immunological reactivity, conformation, etc. Further common characteristics may be physical properties, e.g. migration pattern in gel electrophoresis, chromatographic characteristics, sedimentation coefficient, solubility, spectroscopic properties, stability, pH optimum, temperature optimum, etc..

Polar auxin transport activity can be measured according to Hertel *et al.* (1983) *Planta* 157, 193-201; Parker and Briggs (1990) *Plant Physiol.* 94, 1763-1769; Okada *et al.*, *supra*, Bennett *et al.*, *supra*; general information is also given in Lomax *et al.* (1995) in: Davies (ed.), *Plant Hormones*, Kluwer Dordrecht. Thus, expression of the *pin* gene and activity of the *pin* protein can be easily measured in wild-type, transgenic and mutant plants.

For the preparation of foreign genes for their introduction into higher plants, a variety of cloning vectors are available, e.g. such vectors which have an origin of replication active in *E. coli* and a marker gene for the selection of transformed bacteria cells. Examples for such vectors are pBR322, several pUC-vectors, M13mp-vectors, pACYC184, etc. The desired nucleic acid sequence can be incorporated in a suitable restriction site of the vector and the obtained plasmid is used for the transformation of *E. coli* cells.

Transformed *E. coli* cells are grown in a suitable medium, subsequently harvested and lysed. The plasmid is recovered and the plasmid DNA can now be analyzed and characterized by restriction mapping, gel electrophoresis, sequencing and other biochemical and genetic engineering methods known to the person skilled in the art. After each manipulation the plasmid DNA can be digested and the obtained linearized vectors or fragments can be combined with other DNA sequences. Each plasmid DNA sequence can be cloned in the same or other plasmids.

For introducing DNA into a plant host cell several techniques are available and the person skilled in the art can easily choose a suitable transformation procedure. These techniques comprise the transformation of plant cells with T-DNA using *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* as transformation means, fusion of protoplasts, direct gene transfer of isolated DNA into protoplasts, microinjection or electroporation of DNA, introducing DNA via biolistic methods and other procedures. In an alternative embodiment of the invention the nucleic acid molecules of the invention can be introduced into plant cells via viral infection.

When the DNA is transferred by microinjection or electroporation any form of DNA may be used. The same applies for direct gene transfer. Here, simple plasmids, as for example pUC derivatives can be used. However, if whole plants are to be regenerated from transformed plant cells a marker gene which allows selection of transformed plants is necessary. Suitable selection markers are known to the man skilled in the art and he can easily choose a suitable marker.

Depending on the transformation method used further DNA sequences may be required. For instance, if the Ti- or Ri-plasmid is used, the gene to be transferred has to be combined with at least the right border of the T-DNA contained in the Ti- and Ri-plasmid. Often, the gene of interest has to be combined with both the right and the left border of the T-DNA as flanking areas.

If agrobacteria are used for the transformation, the DNA to be transferred has to be cloned into specific plasmids, i.e. into an intermediary or a binary vector. Intermediary vectors comprise sequences homologous to sequences of the T-DNA which allow them to be integrated in the Ti- or Ri-plasmid of agrobacteria by homologous recombination. Further, this plasmid carries the *vir*-region which is required for the T-DNA transfer. Intermediary vectors are not capable to

replicate in agrobacteria. The intermediary vector can be transferred to agrobacteria via a helper plasmid (conjugation). Binary vectors are able to replicate in both *E. coli* and agrobacteria. They contain a selection marker gene and a linker or polylinker framed by the left and right border regions of the T-DNA. These vectors can be directly transformed into agrobacteria (Holsters *et al.* (1978), Molecular and General Genetics **163**, 181-187). The agrobacterial host cell should contain a plasmid carrying the *vir*-region which is necessary for the transfer of the T-DNA into the plant cell. Addition T-DNA may be present.

The transformed agrobacterium is used for the transformation of plant cells. The use of T-DNA for the transformation of plant cells is well-studied and sufficiently described in EP 120 515; Hoekema in: The Binary Plant Vector System, Offsetdrukkerij Kanters B.V., Alblaserdam (1985) Chapter V; Fraley *et al.* (1993), Crit. Rev. Plant Sci. **4**, 1-46 and An *et al.* (1985), EMBO J. **4**, 277-287.

For the transfer of DNA into plant cells plant explants can be effectively co-cultivated with *Agrobacterium tumefaciens* or *Agrobacterium tumefaciens*. Subsequently, whole plants can be regenerated from the infected plant material (e.g. leaves or leaf pieces, stem segments, roots, protoplasts or plant cells cultivated in suspension cultures) in a suitable medium containing antibiotics or biocides as selection agents. The regeneration of the plants is accomplished by common regeneration methods using known nutrient media. The obtained plants can then be analyzed for the DNA that was to be transferred using standard biochemical and genetic engineering methods. Biolistic transformation methods can also be applied, as described e.g. in Willmitzer (1993) Transgenic Plants, in: Biotechnology, A Multi-Volume Comprehensive Treatise (H.J. Rehm, G. Reed, A. Pühler, P. Stadler, eds) Vol. 2, 627-659, V.C.H. Weinheim - New York - Basel - Cambridge).

While the transformation of dicotyl plants via *A. tumefaciens* using Ti-plasmid-vector systems is well established, recent studies seem to indicate that also monocotyl plants can be transformed via *Agrobacterium*-derived vectors (Chan *et al.* (1993), Plant Mol. Biol. **22**, 491-506; Hiei *et al.* (1994), Plant J. **6**, 271-282; Deng *et al.* (1990), Science in China **33**, 28-34; Wilimink *et al.* (1992), Plant Cell Reports **11**, 76-80; May *et al.* (1995) Bio/Technology **13**, 486-492; Conner and Domiss (1992) Int. J. Plant Sci. **153**, 550-555; Ritchie *et al.* (1993) Transgenic Res. **2**, 252-265).

Alternative systems for the transformation of monocotyledons are procedures using the biolistic approach (Wan and Lemaux (1994), Plant Physiol. **104**, 37-48; Vasil *et al.* (1993), Bio/Technology **11**, 1553-1558; Ritala *et al.* (1994), Plant Mol. Biol. **24**, 317-325; Spencer *et al.* (1990), Theor. Appl. Genet. **79**, 625-631), transformation of protoplasts, electroporation of partially permeabilized cells, the introduction of DNA via glass fibres.

Transformation methods that can be used for the transformation of gymnosperms are described e.g. in Liu *et al.* (1993) Plant Mol. Biol. **23**, 297-308; Primich and Minocha (1991) Plant Cell Reports **10**, 545-549; Morris *et al.* (1989) Phys. Mol. Plant Pathol. **34**, 451-462.

Once the introduced DNA is integrated into the plant genome, it remains stable and is also stably inherited to the progeny of the originally transformed plant cell. Usually, it contains a selection marker conferring resistance against a biocide or antibiotic as kanamycin, G418, bleomycin, hygromycin, methotrexate, glyphosate, streptomycin, sulpho-nyl urea, gentamycin, phosphinotricin, etc. The selection marker which can be chosen individually should therefore allow selection of transformed cells over cells which are devoid of the transferred DNA.

The transformed cells grow within the plant in the normal way (e.g. McCormick *et al.* (1986) Plant Cell Reports **5**, 81-84). The resulting plants can be cultivated in the usual fashion and may be propagated by self-fertilization or be crossed with plants having the same transformed or other hereditary factors. The hybrids obtained by crossings have the respective phenotypic characteristics. Usually, seeds can be obtained from the plants.

Two or more generations should be grown in order to ensure that the phenotypic trait is stably maintained and inherited. Also transgenic lines can be selected by usual methods which are homozygous for the new nucleic acid molecules. Heterozygous and homozygous plants can then be analyzed for their auxin transport and auxin activity and their phenotypic behaviour in respect to vascular tissue development, wood production and structure, juvenility, branching, and other traits which are influenced directly by polar auxin transport and which can be manipulated within the scope of this invention.

The isolation and characterisation of the *pin* genes using a transposon tagging strategy is described in the following examples which are meant to be illustrative but not limiting.

## Description of the Figures

Figure 1 shows the pKEN2 construct what was transformed into the Arabidopsis plants. BL: left border of the T-DNA; BR: right border of the T-DNA; HPT: hygromycin phosphotransferase gene; P35S: Cauliflower Mosaic Virus 35S promoter; P1': TR-DNA 1' promoter; P: En promoter; NPTII: neomycin phosphotransferase II gene (kanamycin resistance gene).

Figure 2 depicts the En insertion site in the *pin1* cDNA.

Figure 3 shows the open reading frame (ORF) of the *pin1* cDNA clone pcpin1/23 (SEQ:NO 11).

Figure 4 depicts the predicted characteristics of the *pin1* protein with respect to the hydrophilic and hydrophobic characteristics and the surface probability. The plots are made by DNASTar, a computer program DNA analysing pro-



# EP 0 814 161 A1

gram using the Kyte-Doolittle and the Emini algorithms.

Figure 5 shows the plasmid map of pcpin1/23. The cDNA of the pin1 gene, contained on a 2.3 kb EcoRI/XhoI fragment was inserted between the EcoRI and XhoI restriction sites of the pBlueScript SK(+/-) vector.

Figure 6 shows the plasmid map of pcpin 2/4. The cDNA of the pin2 gene, contained on a 2.3 kb EcoRI/XhoI fragment was inserted between the EcoRI and XhoI restriction sites of the pBlueScript SK(+/-) vector.

## Sequence list

SEQ:NO 1            En 6988F

5'-GCA GAT GTA TGG AGG TGC CGG AAC-3'

SEQ:NO 2            En 7635R

5'-CGA GTG TAG TAC GAT CAG GCG GAG-3'

SEQ:NO 3            En 578R

5'-AGG TTG GGC AGA AAC ATT TTG CTT-3'

SEQ:NO 4            APL16

5'-phosphorylated-TA TGG GAT CAC ATT AA-3'-amino

SEQ:NO 5            LR 32

5'-ACT CGA TTC TCA ACC CGA AAG TAT AGA TCC CA-3'

SEQ:NO 6            En 205R

(biotin-)5'-AGA AGC ACG ACG GCT GTA GAA TAG GA-3'

SEQ:NO 7            LR 26

5'-ACT CGA TTC TCA ACC CGA AAG TAT AG-3'

SEQ:NO 8 En 91R

5'-TGC AGC AAA ACC CAC ACT TTT ACT TC-3'

SEQ:NO 9 En 48R

5'-TTT ACT TCG ATT AAG AGT GTC GGC C-3'

SEQ:NO 10 En 1R

5'-ACT CCT TTG ACG TTT TCT TGT AGT G-3'

SEQ:NO 11 cDNA sequence of the pin1 gene (pcpin1/23)

1 GTCTCGAGTT TTTTTTCTT TTTTCCCTCT CACCACTTCT CTCTCAAAC  
51 AAAGACAAAA GCTCTTCTCT CTCCTCTCTC TCTTCTCCGG CGAACAAAA  
101 GATGATTACG GCGGCGGACT TCTACCACGT TATGACGGCT ATGGTTCCGT  
151 TATACGTAGC TATGATCCTC GCTTACGGCT CTGTCAAATG GTGGAAAATC  
201 TTCACACCAG ACCAATGCTC CGGCATAAAC CGTTTCGTCTG CTCTCTTCGC  
251 CGTTCCTCTC CTCTCTTTCC ACTTCATCGC CGCTAACAAC CCTTACGCCA  
301 TGAACCTCCG TTTCTCTGCC GCAGATTCTC TCCAGAAAGT CATTGTCCTC  
351 TCTCTCCTCT TCCTCTGGTG CAACTCAGC CGCAACGGTT CTTTAGATTG  
401 GACCATAACT CTCTTCTCTC TCTCGACACT CCCCAACACT CTAGTCATGG  
451 GGATACCTCT TCTCAAAGGC ATGTATGGTA ATTTCTCCGG CGACCTCATG  
501 GTTCAAATCG TTGTTCTTCA GTGTATCATT TGGTACATAC TCATGCTCTT  
551 TCTCTTTGAG TACCGTGGAG CTAAGCTTTT GATCTCCGAG CAGTTTCCAG  
601 ACACAGCAGG ATCTATTGTT TCGATTCTATG TTGATTCCGA CATTATGTCT  
651 TTAGATGGAA GACAACCTTT GGAAACTGAA GCTGAGATTA AAGAAGATGG  
701 GAAGCTTCAT GTTACTGTTC GTCGTTCTAA TGCTTCAAGG TCTGATATTT  
751 ACTCGAGAAG GTCTCAAGGC TTATCTGCGA CACCTAGACC TTCGAATCTA  
801 ACCAACGCTG AGATATATTC GCTTCAGAGT TCAAGAAACC CAACGCCACG  
851 TGGCTCTAGT TTTAATCATA CTGATTTTTTA CTCGATGATG GCTTCTGGTG  
901 GTGGTCGGAA CTCTAACTTT GGTCTGGAG AAGCTGTGTT TGGTTCTAAA

EP 0 814 161 A1

951 GGTCCTACTC CGAGACCTTC CAACTACGAA GAAGACGGTG GTCCTGCTAA  
 1001 ACCGACGGCT GCTGGAAGTG CTGCTGGAGC TGGGAGGTTT CATTATCAAT  
 5 1051 CTGGAGGAAG TGGTGGCGGT GGAGGAGCGC ATTATCCGGC GCCGAACCCA  
 1101 GGGATGTTTT CGCCCAACAC TGGCGGTGGT GGAGGCACGG CGGCGAAAGG  
 1151 AAACGCTCCG GTGGTTGGTG GGAAAAGACA AGACGGAAAC GGAAGAGATC  
 10 1201 TTCACATGTT TGTGTGGAGC TCAAGTGCTT CGCCGGTCTC AGATGTGTTT  
 1251 GGCGGTGGAG GAGGAAACCA CCACGCCGAT TACTCCACCG CTACGAACGA  
 1301 TCATCAAAAG GACGTTAAGA TCTCTGTACC TCAGGGGAAT AGTAACGACA  
 1351 ACCAGTACGT GGAGAGGGAA GAGTTTAGTT TCGGTAACAA AGACGATGAT  
 15 1401 AGCAAAGTAT TGGCAACGGA CGGTGGGAAC AACATAAGCA ACAAACGAC  
 1451 GCAGGCTAAG GTGATGCCAC CAACAAGTGT GATGACAAGA CTCATTCTCA  
 1501 TTATGGTTTG GAGGAAACTT ATTCGTAATC CCAACTCTTA CTCCAGTTTA  
 1551 TTCGGCATCA CCTGGTCCCT CATTTCCCTT AAGTGGAACA TTGAAATGCC  
 20 1601 AGCTCTTATA GCAAAGTCTA TCTCCATACT CTCAGATGCA GGTCTAGGCA  
 1651 TGGCTATGTT CAGTCTTGGG TTGTTCATGG CGTTAAACCC AAGAATAATA  
 1701 GCTTGTGGAA ACAGAAGAGC AGCTTTTGCG GCGGCTATGA GATTTGTCGT  
 25 1751 TGGACCTGCC GTCATGCTCG TTGCTTCTTA TGCCGTTGGC CTCCGTGGCG  
 1801 TCCTCCTCCA TGTTGCCATT ATCCAGGCAG CTTTGCCGCA AGGAATAGTA  
 1851 CCGTTTGTGT TTGCCAAAGA GTATAATGTG CATCCTGACA TTCTTAGCAC  
 1901 TGCGGTGATA TTTGGGATGT TGATCGCGTT GCCCATAACT CTCTCTACT  
 30 1951 ACATTCTCTT GGGTCTATGA AGAGATATTA CCAAACACA GGGACTTTGT  
 2001 TTTATTCTTT TGTGGGATGA TGAATTGTGA AAAGAACAAT GCCCTTTTGT  
 2051 TTGAAAACCC ACAAATTAAA TCAGAAGCAG CTTTAGAGAA TCTTTGAGGA  
 35 2101 TAATTGAAGC TCTTGAAGAA GAGAAGAAGA AGGAGACTTA AGTAGGAGCT  
 2151 CAGCAAGTTT TACCTTTTTC TTAATTTTAA TGAACATTCG TGTTCCTCT  
 2201 TTTGGTAGGT TTTAGGAATT TGTAAGGCT TTGGCTACTT TTAGTGAATT  
 40 2251 AAAAACGTTA AGGAAAATAT CAAAAA AAAA AAAA AA

SEQ:NO 12 cDNA sequence of the pin2 gene (pcpin2/4)

45 1 GGCACGAGCC GGTTCGTTGC GGTTTTGCGG GTTCCTCTTC TCTCTTTCCA  
 51 TTTTCATCTCC TCCAATGATC CTTATGCAAT GAATTACCAC TTCCTCGCTG  
 101 CTGATTCTCT TCAGAAAGTC GTTATCCTCG CCGCACTCTT TCTTTGGCAG  
 50 151 GCGTTTAGCC GCAGAGGAAG CCTAGAATGG ATGATAACGC TCTTTTCACT  
 201 ATCAACACTG CCTAACACGT TGTAATGGG AATCCCATTG CTTAGGGCGA

251 TGTACGGAGA CTTCTCCGGT AACCTAATGG TGCAGATCGT GGTGCTTCAG  
301 AGCATCATAT GGTATACATT AATGCTCTTC TTGTTTGAGT TCCGTGGGGC  
5 351 TAAGCTTCTC ATCTCCGAGC AGTTCCTCGA GACGGCTGGT TCAATTACTT  
401 CCTTCAGAGT TGAATCTGAT GTTATCTCTC TTAATGGCCG TGAACCCCTC  
451 CAGACCGATG CGGAGATAGG AGACGACGGA AAGCTACACG TGGTGGTTCG  
10 501 AAGATCAAGT GCCGCCTCAT CAATGATCTC TTCATTCAAC AAATCTCACG  
551 GCGGAGGACT TAACTCCTCC ATGATAACGC CGCGAGCTTC AAATCTCACG  
601 GGCGTAGAGA TTTACTCCGT TCAATCGTCA CGAGAGCCGA CGCCGAGAGC  
651 TTCTAGCTTT AATCAGACAA ATTTCTACGC AATGTTTAAC GCAAGCAAAG  
15 701 CTCCAAGCCC TCGTCACGGT TACACTAATA GCTACGGCGG CGCTGGAGCT  
751 GGTCCAGGTG GAGATGTTTA CTCACTTCAG TCTTCTAAAG GCGTGACGCC  
801 GAGAACGTCA AATTTTGATG AGGAAGTTAT GAAGACGGCG AAGAAAGCAG  
20 851 GAAGAGGAGG CAGAAGTATG AGTGGGGAAT TATACAACAA TAATAGTGTT  
901 CCGTCGTACC CACCGCCGAA CCAATGTTC ACGGGGTCAA CGAGTGGAGC  
951 AAGTGGAGTC AAGAAAAAGG AAAGTGGTGG CGGAGGAAGC GGTGGCGGAG  
1001 TAGGAGTAGG AGGACAAAAC AAGGAGATGA ACATGTTCGT GTGGAGTTCG  
25 1051 AGTGCTTCTC CGGTGTCGGA AGCCAACGCG AAGAATGCTA TGACCAGAGG  
1101 TTCTTCCACC GATGTATCCA CCGACCCTAA AGTTTCTATT CCTCCTCACG  
1151 ACAACCTCGC TACTAAAGCG ATGCAGAATC TGATAGAGAA CATGTCACCG  
1201 GGAAGAAAAG GGCATGTGGA AATGGACCAA GACGGTAATA ACGGGGGAAA  
30 1251 GTCACCTTAC ATGGGCAAAA AAGGTAGCGA CGTGGAAGAC GGCGGTCCCG  
1301 GTCCTAGGAA ACAGCAGATG CCGCCGGCGA GTGTGATGAC GAGACTAATT  
1351 CTGATAATGG TTTGGAGAAA ACTCATTCGA AACCCTAACA CTTACTCTAG  
35 1401 TCTCTTTGGC CTTGCTTGGT CCCTTGCTC TATCAAGTGG AATATAAAGA  
1451 TGCCAACGAT AATGAGTGGG CCGATTTCTGA TATTATCTGA TGCTGGTCTT  
1501 GGAATGGCTA TGTTTAGTCT TGGTCTATTT ATGGCATTGC AACCAAAGAT  
40 1551 TATTGCGTGC GGAAAATCAG TAGCAGGGTT TGCATGGCC GTAAGGTCTT  
1601 TGACTGGACC AGCCGTGATC GCAGCCACCT CAATAGCAAT TGGTATTCTGA  
1651 GGTGATCTCC TCCATATCGC CATCGTTTCTG GCTGCTCTTC CTCAAGGAAT  
1701 CGTTCCTTTT GTTTTCGCCA AAGAATATAA CGTCCATCCT GATATTCTCA  
45 1751 GCACTGCGGT TATATTCGGA ATGCTGGTTG CTTTGCCTGT AACAGTACTC  
1801 TACTACGTTT TTTTGGGGCT TTAAGTTATT ATCAAAACGT ATTTGCAAAT  
1851 AAAAGGCGAT ACGACCCAAA GGTGATTTTT TTTCAAACGA AAAAGAATAA  
50 1901 TTACAAGAAC GAAAAAGAC TAATTCCAGG TCAGAGGCTT AGGTGTATGG  
1951 GACCATGCAA TGTCGCATTA ATTAAATTAT AGCATATGAT AGTCGAAAAT  
2001 TTAGATAACT TTGTATAATT AATTATATGC ACATGCATGT ACGTGACTTT

2051 GTAGTTTTTG TTACATTTAT TAAATTTTTG GGATGTGCAA GTACAATTAT  
 2101 TACTAAAAAA AAAAAAA

5

## EXAMPLE I

10

Generation of En transposon mutagenised *Arabidopsis thaliana* population

1. Vector for introduction of the heterologous transposon En into *Arabidopsis thaliana*

15 The heterologous En transposon (described in Pereira *et al.* (1986), EMBO J. 5, 835-841) was integrated into the genome of *Arabidopsis thaliana* using the agrobacterial plant transformation system. pKen2 (Fig. 1) was the binary transformation vector that carried the En transposable element on the T-DNA. Detailed information about the plasmid pKen2 it given in Frey *et al.* (1990), EMBO J. 9, 4037-4044 and Cardon *et al.* (1993), Plant J. 3, 773-784).

20 2. Plant material and transformation

Roots of *A. thaliana* (L.) Heynh ecotype Columbia (Columbia "0") were transformed with pKen2 by *Agrobacterium tumefaciens* using the protocol described by Valvekens *et al.* (1988), Proc. Natl. Acad. Sci. USA 85, 5536-5540. Root explants were obtained from plants growing axenically on solid or liquid GM medium (Valvekens *et al. supra*). For cul-  
 25 ture in liquid medium, 2-week-old seedlings were transferred to 250 ml Erlenmayer flasks containing 75 ml GM medium and incubated on a rotatory shaker at 100 r.p.m. for 3 weeks. Growth of *A. tumefaciens* after co-cultivation was inhibited with 400 mg/l claforan (available from Hoechst, Frankfurt, Germany). This amount was reduced stepwise with every passage to fresh medium (minus 100 mg/l every 2 weeks) until a final concentration of 100 mg/l was reached. Trans-  
 30 formed cells, as well as the progeny of transformed plants, were selected with 50 mg/l kanamycin monosulphate (available from Sigma, München, Germany), 15 mg/l hygromycin B (available from Duchefa, Haarlem, Netherlands) or 50 µg/l methotrexate (available from Sigma, München, Germany). The number of transformed loci was estimated by the segregation ratio of the marker gene linked to the T-DNA and later confirmed by Southern analysis (Sambrook *et al. supra*).

35 3. Origin of the En *pin1* mutant plant material

T0:

- 40 - *A. thaliana* ecotype Columbia "0". Root material of this plant was transformed with *A. tumefaciens* as described above. The binary transformation vector carrying the En transposon on its T-DNA was pKen2 (see Fig. 1).

T1:

- 45 - 24-2A, single copy primary transformant, hygromycin (hyg) resistant

T2:

- 50 - self of 24-2A  
 - 26 plants homozygous for hyg resistance (NPTII::En/NPTII::En),  
 - 20 plants hemizygous for hyg resistance (NPTII::En/wt) (wt = wild-type)  
 - about 7.5 % were germinal revertants carrying a transposon En or not

T3:

- 55 - selfs (about 3900 plants) of the 46 T2 plants  
 - selection on 300 to 400 mg/l kanamycin (km) and screening for germinal revertants (350 revertants)  
 - genotypes NPTII::En/NPTII, NPTII/NPTII or NPTII/wt  
 - germinal excision of En in *Arabidopsis* it about 7.5 %



T4:

- selfs of 350 revertants of T3 (100 to 200 seeds per revertant)
- screen for insertion mutants, transposed and newly inserted Ens (Southern blot analysis)
- cross to wild-type Arabidopsis using pollen from the revertant plants 293, 325, 328, 330 to outcross the T-DNA, aim: outcross of T-DNA, the transposon remaining active in the plant

T5:

- F1 generation of the cross: wt x 292 / 325 / 328 / 330,
- PCR screen for plants carrying a transposed En and no T-DNA, but the plants had all the NPTII gene (tEn/wt, NPTII/wt),
- another selfing

T6/S0:

- selfs of T5 plants = F2 generation of the cross: wt. x germinal revertants
- PCR screen for plants carrying a transposed En and no T-DNA
- positive plants: G11 (line 325)  
G24, G37, G38 (line 328)  
G48, G54 (line 330)  
G69 (line 293)
- genotype: tEn/wt or tEn/tEn, no NPTII gene

S1:

- 100 selfs of G11, G24, G37, G48, and G69 (100 seeds of each plant sown, in total 500 S1 plants)

S2:

- single seed descendents of the 500 selfed plants of S1

S3:

- single seed descendents of the 500 selfed plants of S2

S4:

- single seed descendents of the 500 selfed plants of S3

S5:

- single seed descendents of the 500 selfed plants of S4

S6:

- single seed descendents of the 500 selfed plants of S5
- 150 plants analysed on Southern blot

S7:

- 14 seeds (selfs) of 70 G24 S6 single seed descendents sown
- in one out of the 70 (14 seed) pools, the one of which the parent plant is 5673-2, the En pin1 mutant is detected
- > 5673-2 (S6) is hemizygous for the pin1 mutation

## EXAMPLE II

Screening and identification of a pin1 mutant in the En mutagenised population1. Detection and identification of the pin1 mutant: characteristics

The pin1 mutant was detected by its obvious, morphological phenotype, that has already described in literature (Okada *et al.*, *supra*). The detected En transposon pin1 mutant exhibited a striking similarity to the pin-formed 1-1 mutant, derived from the Enkheim ecotype and to the pin-formed 1-2 mutant, isolated from ethyl methanesulfonate (EMS) mutagenised seeds of the Landsberg ecotype (Okada *et al.*, *supra*). As these mutants the En transposon pin1 mutant showed structural and morphological abnormalities in the inflorescence axes, flowers, leaves and cotyledons. The most obvious phenotype is the pin-shaped inflorescence with no flowers or floral buds at all or few deformed and sterile flowers, or just some pistil like tissue on top of it. When flowers or flower like organs are formed, the petals are abnormally shaped, no stamens with pollen are formed and the pistil is usually formed with abundant papillae tissue, but fertilisation can not take place. Some of the leaves of that pin1 mutant become much wider and attain a quite deformed and distorted shape. In some cases the major vein is branched at the base of the leaf. Some transposon pin1 mutants have three cotyledons whereas in other cases the cotyledons are fused or deformed.

2. Mendelian Segregation of the pin1 phenotype

126 siblings (S7 of 5673-2) of the identified En transposon pin1 mutant were sown out in the greenhouse and the following segregation pattern was observed:

95 wild type : 31 pin1 phenotype (3,06 : 1,0)

This ratio approximates quite reasonably the Mendelian segregation ratio of 3:1 for the inheritance of a recessive genetic trait in a selfing experiment. The fact that the pin1 mutation is recessive has already been reported in the literature.

3. Southern blot analysis of the pin1 segregating population using En specific probes

Genomic DNA of 37 plants of the pin1 phenotype segregating population (12 pin1 phenotypes and 25 wild-type phenotypes) was applied to Southern hybridization experiments using a right end and a left end radioactively labelled probe from the En transposon base pair sequence. The En element contains two XbaI restriction sites, one around 800 bp from the 5' end (left end) and the other about 2,000 bp from the 3' end (right end). The genomic DNA was therefore restricted with XbaI and En elements integrated into the plant genomes could be detected by 2 specific probes:

- En right end probe: En DNA amplified by PCR with primers:  
En 6988F (SEQ NO:1) and En 7635R (SEQ NO:2).
- En left end probe: En DNA amplified by PCR with primers:  
En 578R (SEQ NO:3) and universal primer (available from Pharmacia, Freiburg, Germany).

## Results:

- integration of several (2-7) transposons into the Arabidopsis genome
- among the 25 wild-type phenotypes 15 plants are heterozygous for the En mutated pin1 allele
- correlation between the pin1 phenotype and 1 En band (pin1 typic) indicating that the mutant is transposon tagged

## EXAMPLE III

## Isolation of the En flanking DNA by the transposon insertion display (TID) procedure

1. Digestion and purification of pin1 mutant genomic DNA

To isolate the flanking DNA of the pin1 typic En element 1 µg of genomic DNA of Enpin1 mutant plants was digested with the restriction enzyme Csp6I (available from New England Biolabs, Schwalbach, Germany) in parallel with wild-type plants as controls. The genomic plant DNA flanking the left end (5' end) of the pin1 typic En transposon was isolated via a linker mediated PCR procedure. The Csp6I restriction digest produced a DNA fragment with a convenient size of about 900 bp for PCR amplification. After the digest the DNA was purified with QIAquick-spin PCR Purification Kit (available from Qiagen, Hilden, Germany).

## 2. Ligation of adapters to fragmented genomic DNA

200 ng of the Csp6I digested and purified DNA were used in the ligation to 50 pmol APL1632 linker. APL1632 is a partial annealing product of 2 oligos: APL16 (SEQ NO:4) and LR32 (SEQ NO:5). Annealing of these 2 oligos results in a TA overhang that can be ligated to the Csp6I sticky end. The ligation was carried out with the T4 DNA Ligase and the ligation buffer from Promega Serva, Heidelberg, Germany, at 16 °C over night. After ligation the purification was repeated with the Qiaquick-spin PCR Purification Kit.

## 3. Biotinylated En primer extension reactions

Primer extension reactions were carried out on the adapter linked genomic DNA fragments using the En specific primer En 205R (SEQ NO:6). This primer was biotinylated in order to select specifically synthesized molecules of that reaction. The reaction mixture was as follows:

DNA with linkers (50 ng)	12.5 µl
10x PCR buffer	5.0 µl
10x dNTPs (2mM)	5.0 µl
20x TMAC (1mM) (tetra methyl ammonium chloride)	2.5 µl
biotinylated En 205R	10 pmol
Taq Polymerase (from Boehringer, Mannheim, Germany)	2.5 Units
water	to 50 µl

The reaction covered with mineral oil was done on a thermocycler TRIO-block with the following program (12 cycles):

90 °C	30 sec
94.5 °C	35 sec
64 °C	90 sec
73 °C	300 sec
4 °C	pause

The QIAquick-spin purification was repeated and the DNA eluted with 50 µl 10 mM Tris/HCl, pH 8.0.

## 4. Specific binding to Dynabeads M-280 streptavidine coated beads

To get rid of the non template DNA M-280 streptavidine coated beads (Dyna, Oslo, Norway) were used. 50 µl 2M NaCl were added to the purified DNA solution (50 µl). The Dynabeads were washed and resuspended in the original volume of B&W buffer (2 mM NaCl/TE, pH 7.6) and 10 µl Dynabeads solution were added to 100 µl of DNA solution. The biotinylated En 205R primer specific DNA molecules were bound to Dynabeads M-280 streptavidine coated beads during incubation for 30 minutes at room temperature. The beads were washed using the Dynal MPC magnet as follows:

2 x 200 µl B&W buffer  
2 x 200 µl TE, pH 7.6 / 0.01 % Tween 20.

Then the beads were resuspended in 200 µl TE, pH 7.6 / 0.01 % Tween 20 and stored at 4 °C.

## 5. PCR preamplification

40 µl of the DNA-Dynabeads complex with the buffer removed on the magnet were taken for the following PCR

# EP 0 814 161 A1

reaction (25  $\mu$ l):

10x buffer	2.5 $\mu$ l
10x dNTPs (2 mM)	2.5 $\mu$ l
20x TMAC (1 mM)	1.25 $\mu$ l
LR26 linker primer (SEQ:NO 7)	25 pmol
En 91R (SEQ:NO 8)	25 pmol
Tag polymerase (Boehringer)	0.25 Units
water	to 25 $\mu$ l

The following PCR program (30 cycles) was run:

82 °C	pause
94 °C	35 sec
64 °C	60 sec
73 °C	90 sec
73 °C	180 sec
4 °C	pause

During the 82 °C pause the Tag polymerase was added under the oil (hot start).

## 6. Primer extensions with radioactively labelled primer

PCR amplified DNA fragments were used as primer extension reactions. En primers used in these reactions were radioactively labeled with gamma-<sup>32</sup>P-ATP by T4 polynucleotide kinase (from New England Biolabs, Schwalbach, Germany. For one radioactive primer extension reaction in 10  $\mu$ l a mixture was made of

preamplification reaction	1.6 $\mu$ l
10x buffer	0.84 $\mu$ l
10x dNTPs	0.84 $\mu$ l
20x TMAC	0.42 $\mu$ l
labelled primer En 48R (SEQ:NO 9)	0.75 pmol
Tag polymerase (Boehringer)	0.05 Units
Water	to 10 $\mu$ l

The reaction mix was incubated on a thermocycler running the following program:

82 °C	60-120 sec
94 °C	35 sec
64 °C	60 sec
73 °C	90 sec
73 °C	180 sec
4 °C	pause

25 cycles of primer extension reaction were carried out. 6 µl of DNA sequencing stop solution (Promega) were added afterwards.

#### 7. Separation of the radioactively labelled DNA on a polyacrylamide gel and excision of the fragment of interest

2-4 µg of the primer extension products as well as radioactively labelled 10 bp DNA ladder (Sequamar, Research Genetics, Inc., Huntsville, AL, USA) were denatured and loaded on a 6 % polyacrylamide gel electrophoresis (PAGE). After separation of the DNA the gel was exposed to a X-ray (without fixation) to detect the radioactive bands and their molecular size. The radioactive band of the flanking DNA of the pin1 typic En insertion was detected by its predicted molecular size. The gel fragment containing this DNA of interest was cut out and incubated in 50 µl TE, pH 8.0, to make the DNA diffuse out of the gel slice. The DNA eluted from the gel was PCR amplified using the En specific primer En 48R (SEQ:NO 9) and the adapter specific primer LR26 (SEQ:NO 7). In order to make sure that really flanking genomic DNA of an En element was isolated and to obtain its DNA sequence cycling sequencing was carried out with the excised and PCR amplified DNA. Cycling sequencing was carried out with the Promega fmol DNA Sequencing System using a gamma-<sup>32</sup>P-ATP end-labelled En 48R (SEQ:NO 9) primer. The obtained DNA sequence showed the transition of the En left end sequence to genomic DNA proving that En flanking DNA had been isolated. To show that the flanking DNA of the pin1 typic En element has been isolated the prepared Southern blots were hybridized with a flanking DNA probe synthesized by PCR on the isolated DNA using the primers En 1R (SEQ:NO 10) and LR26 (SEQ:NO 7).

#### EXAMPLE IV

##### Cloning the complete pin1 cDNA

To get the full size cDNA of the pin1 gene a lambda phage cDNA library of an Arabidopsis thaliana suspension culture was screened. The cDNA library was prepared in the Uni-ZAP XR vector of the lambda ZAPII system from Stratagene, La Jolla, CA, USA and Heidelberg, Germany). The radioactively labelled flanking DNA that had been PCR amplified using the primers En 1R (SEQ:NO 10) and LR 26 (SEQ:NO 7) served as probe for the phage screening. In the second plating positive signals could be detected from single phage plaques. The pBluescript phagemid carrying the pin1 cDNA was obtained from the positive lambda phages in Escherichia coli using the in vivo excision protocol of the ZAP-cDNA Gigapack II Gold Cloning Kit (Stratagene). The excised pBluescript vectors carrying pin1 cDNAs (pcpin1) were checked on a Southern blot with the same probe that was used for the isolation of the single lambda phages.

#### EXAMPLE V

##### Sequence analysis of the pin1 cDNA clones

##### 1. Base pair sequence of the cDNA clone pcpin1/23

The DNA sequence of the cDNA clone pcpin1/23 (SEQ:NO 11) was obtained by the alignment of sequences from deletion clones of pcpin1/23. The deletion clones produced by exonuclease III (New England Biolabs, Schwalbach, Germany) reactions were sequenced on the automated ALF sequencer from Pharmacia. Mapping the isolated flanking



DNA of the *pin1* typical *En* insertion on the sequence of the *pin1* cDNA shows the insertion site of the *En* transposon on the level of the cDNA. Figure 2 depicts the insertion of the *En* element in the *pin1* cDNA.

## 2. Open reading frame

The open reading frame given by DNA computer programs (GCG, University of Wisconsin Computer group, USA; DNA Star) is shown in Figure 3. The most obvious open reading frame is starting from an ATG at position 101 extending to the stop codon TGA at position 1966.

## 3. Predicted characteristics of the *pin1* protein

The hydrophilic and hydrophobic characteristics of a protein encoded by the open reading frame of the *pin1* cDNA and the surface probability are shown in Figure 4. The plots are made by DNASTar, a computer DNA analysing program using the Kyte-Doolittle and the Emini algorithms. The translated open reading frame shows a protein of 622 amino acids, that has a quite hydrophilic middle part, whereas the 5' and 3' ends consist of several hydrophobic domains. The surface probability follows that triple division of the protein.

### Example VI

## Mapping the genomic position of the *pin1* gene

### 1. Mapping on the CIC YAC library

The CIC library is a genomic library of *Arabidopsis thaliana* (ecotype Colombia) in Yeast Artificial Chromosomes (YAC). It consists of 1152 clones with an average insert size of 420 kb. The library represents around 4 nuclear genome equivalents (Creusot et al. (1995), The Plant J. 8, 763-770). The nitrocellulose filters on which the CIC library YAC clone DNA were arrayed were hybridized with the radioactively labelled full size *pin1* cDNA. The autoradiogram showed besides several clones with fainter signals four YAC clones with an equally intensive signal. The YAC clone numbers are the following:

CIC6H1  
CIC9C4  
CIC12H9  
CIC2F9.

On the physical genome map of *Arabidopsis thaliana* all four clones could be localized close together at the bottom of chromosome 1 between the markers *nga111* and *m532*. Bennett *et al.* (1995), The Plant J. 8, 505-520, mapped the *pin*-formed locus (*pin1* gene) in the same chromosomal region on the genetic map of *Arabidopsis thaliana* close to the *crabs claw* locus.

### 2. Allelic test

In order to check whether the *En pin1* mutation in *Arabidopsis thaliana* is allelic to the *pin*-formed 1-1 mutation (Okada *et al.*, supra), both mutants heterozygous for the *pin1* mutation were crossed. The F1 generation showed *pin1* phenotypes and wild type phenotypes in a ratio that approximated 1:3, what is expected for a Mendelian segregation of two recessive alleles in a crossing. Therefore the *En pin1* mutation and the *pin*-formed 1-1 mutations (Okada *et al.*, supra) are allelic.

The cDNA of the *pin2* gene from *A. thaliana* (SEQ:NO 12, *pcpin2/4*) was obtained by the same procedure as described above for the cDNA of the *pin1* gene.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

## (i) APPLICANT:

- (A) NAME: Max-Planck-Gesellschaft zur Foerderung der  
Wissenschaften e.V.  
(B) STREET: Hofgartenstrasse 2  
(C) CITY: Muenchen  
(E) COUNTRY: Germany  
(F) POSTAL CODE (ZIP): 80539

(ii) TITLE OF INVENTION: Genetic control of polar auxin transport in  
plants and manipulation of plant growth, architecture and  
morphogenesis

(iii) NUMBER OF SEQUENCES: 12

## (iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

## (2) INFORMATION FOR SEQ ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GCAGATGTAT GGAGGTGCCG GAAC

24

## (2) INFORMATION FOR SEQ ID NO: 2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

CGAGTGTAGT ACGATCAGGC GGAG

24

## (2) INFORMATION FOR SEQ ID NO: 3:

## (i) SEQUENCE CHARACTERISTICS:

EP 0 814 161 A1

(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: singl  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid  
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

AGGTTGGGCA GAAACATTTT GCTT

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 16 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid  
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

TATGGGATCA CATTAA

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 32 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid  
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

ACTCGATTCT CAACCCGAAA GTATAGATCC CA

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 26 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid  
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

AGAAGCACGA CGGCTGTAGA ATAGGA

26

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

ACTCGATTCT CAACCCGAAA GTATAG

26

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

TGCAGCAAAA CCCACACTTT TACTTC

26

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

TTTACTTCGA TTAAGAGTGT CGGCC

25

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

# EP 0 814 161 A1

(ii) MOLECULE TYPE: other nucleic acid  
(A) DESCRIPTION: /desc = "oligonucleotide"

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

ACTCCTTTGA CGTTTTCTTG TAGTG

25

10

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2292 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: cDNA to mRNA

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GTCTCGAGTT TTTTTTCTT TTTTCCCTCT CACCACTTCT CTCTCAAAC	AAAGACAAAA	60
GCTCTTCTCT CTCCCTCTC TCTTCTCCGG CGAACAAAA	GATGATTACG GCGGCGGACT	120
TCTACCACGT TATGACGGCT ATGGTTCCGT TATACGTAGC TATGATCCTC	GCTTACGGCT	180
CTGTCAAATG GTGGAAAATC TTCACACCAG ACCAATGCTC CGGCATAAAC	CGTTTCGTCG	240
CTCTCTTCGC CGTTCCTCTC CTCTCTTTCC ACTTCATCGC CGCTAACAAAC	CCTTACGCCA	300
TGAACCTCCG TTTCTCTGCC GCAGATTCTC TCCAGAAAGT CATTGTCCCTC	TCTCTCTCTCT	360
TCCTCTGGTG CAAACTCAGC CGCAACGGTT CTTTAGATTG GACCATAACT	CTCTTCTCTC	420
TCTCGACACT CCCCAACACT CTAGTCATGG GGATACCTCT TCTCAAAGGC	ATGTATGGTA	480
ATTTCTCCGG CGACCTCATG GTTCAAATCG TTGTTCTTCA GTGTATCATT	TGGTACATAC	540
TCATGCTCTT TCTCTTTGAG TACCGTGGAG CTAAGCTTTT GATCTCCGAG	CAGTTTCCAG	600
ACACAGCAGG ATCTATTGTT TCGATTCTAG TTGATTCCGA CATTATGTCT	TTAGATGGAA	660
GACAACTTTT GGAAACTGAA GCTGAGATTA AAGAAGATGG GAAGCTTCAT	GTTACTGTTC	720
GTCGTTCTAA TGCTTCAAGG TCTGATATTT ACTCGAGAAG GTCTCAAGGC	TTATCTGCGA	780
CACCTAGACC TTCGAATCTA ACCAACGCTG AGATATATTC GCTTCAGAGT	TCAAGAAACC	840
CAACGCCACG TGGCTCTAGT TTTAATCATA CTGATTTTFA CTCGATGATG	GCTTCTGGTG	900
GTGGTCGGAA CTCTAACTTT GGTCTGGAG AAGCTGTGTT TGGTTCTAAA	GGTCCTACTC	960
CGAGACCTTC CAACTACGAA GAAGACGGTG GTCCTGCTAA ACCGACGGCT	GCTGGAAC TG	1020
CTGCTGGAGC TGGGAGGTTT CATTATCAAT CTGGAGGAAG TGGTGGCGGT	GGAGGAGCGC	1080
ATTATCCGGC GCCGAACCCA GGGATGTTTT CGCCCAACAC TGGCGGTGGT	GGAGGCACGG	1140
CGGCGAAAGG AAACGCTCCG GTGGTTGGTG GGAAAAGACA AGACGGAAAC	GGAAGAGATC	1200

55



EP 0 814 161 A1

TTCACATGTT TGTGTGGAGC TCAAGTGCTT CGCCGGTCTC AGATGTGTTC GGCGGTGGAG 1260  
 GAGGAAACCA CCACGCCGAT TACTCCACCG CTACGAACGA TCATCAAAAG GACGTTAAGA 1320  
 5 TCTCTGTACC TCAGGGGAAT AGTAACGACA ACCAGTACGT GGAGAGGGAA GAGTTTAGTT 1380  
 TCGGTAACAA AGACGATGAT AGCAAAGTAT TGGCAACGGA CGGTGGGAAC AACATAAGCA 1440  
 ACAAACGAC GCAGGCTAAG GTGATGCCAC CAACAAGTGT GATGACAAGA CTCATTCTCA 1500  
 10 TTATGGTTTG GAGGAAACTT ATTCGTAATC CCAACTCTTA CTCCAGTTTA TTCGGCATCA 1560  
 CCTGGTCCCT CATTTCCCTC AAGTGGAAAC TTGAAATGCC AGCTCTTATA GCAAAGTCTA 1620  
 TCTCCATACT CTCAGATGCA GGTCTAGGCA TGGCTATGTT CAGTCTTGGG TTGTTCATGG 1680  
 15 CGTTAAACCC AAGAATAATA GCTTGTGGAA ACAGAAGAGC AGCTTTTGCG GCGGCTATGA 1740  
 GATTTGTTCG TGGACCTGCC GTCATGCTCG TTGCTTCTTA TGCCGTTGGC CTCCGTGGCG 1800  
 TCCTCCTCCA TGTTGCCATT ATCCAGGCAG CTTTGCCGCA AGGAATAGTA CCGTTTGTGT 1860  
 20 TTGCCAAAGA GTATAATGTG CATCCTGACA TTCTTAGCAC TGCGGTGATA TTTGGGATGT 1920  
 TGATCGCGTT GCCCATAACT CTTCTCTACT ACATTCTCTT GGGTCTATGA AGAGATATTA 1980  
 CCAAAACACA GGGACTTTGT TTTATTCTTT TGTGGGATGA TGAATTGTGA AAAGAACAAT 2040  
 25 GCCCTTTTGG TTGAAAACCC ACAAATTTAA TCAGAAGCAG CTTTAGAGAA TCTTTGAGGA 2100  
 TAATTGAAGC TCTTGAAGAA GAGAAGAAGA AGGAGACTTA AGTAGGAGCT CAGCAAGTTT 2160  
 TACCTTTTTC TTAATTTTAA TGAACATTCG TGTTTCCTCT TTTGGTAGGT TTTAGGAATT 2220  
 TGTAAGAGCT TTGGCTACTT TTAGTGAATT AAAAACGTTA AGGAAATAT CAAAAAATAA 2280  
 30 AAAAAAAAAA AA 2292

(2) INFORMATION FOR SEQ ID NO: 12:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 2117 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GGCACGAGCC GGTTCGTTGC GGTTTTCGCG GTTCCTCTTC TCTCTTTCCA TTTCATCTCC 60  
 45 TCCAATGATC CTTATGCAAT GAATTACCAC TTCCTCGCTG CTGATTCTCT TCAGAAAGTC 120  
 GTTATCCTCG CCGCACTCTT TCTTTGGCAG GCGTTTAGCC GCAGAGGAAG CCTAGAATGG 180  
 ATGATAACGC TCTTTTCACT ATCAACACTG CCTAACACGT TGGTAATGGG AATCCCATTG 240  
 50 CTTAGGGCGA TGTACGGAGA CTTCTCCGGT AACCTAATGG TGCAGATCGT GGTGCTTCAG 300  
 AGCATCATAT GGTATACATT AATGCTCTTC TTGTTTGAGT TCCGTGGGGC TAAGCTTCTC 360

EP 0 814 161 A1

	ATCTCCGAGC AGTTCCTCGGA GACGGCTGGT TCAATTACTT CCTTCAGAGT TGA	420
5	GTTATCTCTC TTAATGGCCG TGAACCCCTC CAGACCGATG CGGAGATAGG AGACGACGGA	480
	AAGCTACACG TGGTGGTTCG AAGATCAAGT GCCGCCTCAT CAATGATCTC TTCATTCAAC	540
	AAATCTCACG GCGGAGGACT TAACTCCTCC ATGATAACGC CGCGAGCTTC AAATCTCACC	600
10	GGCGTAGAGA TTTACTCCGT TCAATCGTCA CGAGAGCCGA CGCCGAGAGC TTCTAGCTTT	660
	AATCAGACAA ATTTCTACGC AATGTTTAAC GCAAGCAAAG CTCCAAGCCC TCGTCACGGT	720
	TACACTAATA GCTACGGCGG CGCTGGAGCT GGTCCAGGTG GAGATGTTTA CTCACTTCAG	780
15	TCTTCTAAAG GCGTGACGCC GAGAACGTCA AATTTTGATG AGGAAGTTAT GAAGACGGCG	840
	AAGAAAGCAG GAAGAGGAGG CAGAAGTATG AGTGGGGAAT TATACAACAA TAATAGTGTT	900
	CCGTCGTACC CACCGCCGAA CCAATGTTC ACGGGGTCAA CGAGTGGAGC AAGTGGAGTC	960
20	AAGAAAAAGG AAAGTGGTGG CGGAGGAAGC GGTGGCGGAG TAGGAGTAGG AGGACAAAAC	1020
	AAGGAGATGA ACATGTTTCT GTGGAGTTCG AGTGCTTCTC CGGTGTCGGA AGCCAACGCG	1080
	AAGAATGCTA TGACCAGAGG TTCTTCCACC GATGTATCCA CCGACCTAA AGTTTCTATT	1140
25	CCTCCTCACG ACAACCTCGC TACTAAAGCG ATGCAGAATC TGATAGAGAA CATGTCACCG	1200
	GGAAGAAAAG GGCATGTGGA AATGGACCAA GACGGTAATA ACGGGGAAA GTCACCTTAC	1260
	ATGGGCAAAA AAGGTAGCGA CGTGAAGAC GCGGTCCCG GTCCTAGGAA ACAGCAGATG	1320
30	CCGCCGCGA GTGTGATGAC GAGACTAATT CTGATAATGG TTTGGAGAAA ACTCATTCGA	1380
	AACCCTAACA CTACTCTAG TCTCTTTGGC CTGTCTTGGT CCCTTGTCTC TATCAAGTGG	1440
	AATATAAAGA TGCCAACGAT AATGAGTGA CCGATTTCTGA TATTATCTGA TGCTGGTCTT	1500
35	GGAATGGCTA TGTTTAGTCT TGGTCTATTT ATGGCATTGC AACCAAGAT TATTGCGTGC	1560
	GGAAAATCAG TAGCAGGGTT TGGCATGGCC GTAAGGTTCT TGA	1620
	GCAGCCACCT CAATAGCAAT TGGTATTCGA GGTGATCTCC TCCATATCGC CATCGTTCAG	1680
40	GCTGCTCTTC CTCAAGGAAT CGTTCCTTTT GTTTTCGCCA AAGAATATAA CGTCCATCCT	1740
	GATATTCTCA GCACTGCGGT TATATTCGGA ATGCTGGTTG CTTTGCCTGT AACAGTACTC	1800
	TACTACGTTT TTTTGGGGCT TTAAGTTATT ATCAAAACGT ATTTGCAAAT AAAAGGCGAT	1860
45	ACGACCCAAA GGTGATTTTT TTTCAAACGA AAAAGAATAA TTACAAGAAC GAAAAAGAC	1920
	TAATTCCAGG TCAGAGGCTT AGGTGTATGG GACCATGCAA TGTCGCATTA ATTAAATTAT	1980
	AGCATATGAT AGTCGAAAAT TTAGATAACT TTGTATAATT AATTATATGC ACATGCATGT	2040
50	ACGTGACTTT GTAGTTTTTG TTACATTTAT TAAATTTTTG GGATGTGCAA GTACAATTAT	2100
	TACTAAAAAA AAAAAA	2117

55

Claims

1. DNA sequences that code for a pin protein or an active fragment thereof, and the alleles as well as derivatives of these DNA sequences.
2. DNA sequences according to claim 1 isolated from plants.
3. DNA sequences according to claim 1 or 2 wherein said protein is involved in polar auxin transport and/or auxin activity.
4. DNA sequences according to one of the preceding claims wherein said protein is essential for polar auxin transport activity and/or auxin activity.
5. DNA sequences according to one of the preceding claims wherein said protein is located to the plasma membrane and/or associated with the cytoskeleton.
6. DNA sequences according to one of the preceding claims wherein said protein is an auxin receptor protein and/or an auxin carrier protein and/or in contact with an auxin receptor protein and/or an auxin carrier protein.
7. DNA sequences according to one of the preceding claims wherein said protein controls the activity of the auxin efflux carrier proteins or of other proteins involved in polar auxin transport.
8. DNA sequences according to one of the preceding claims wherein said protein is involved in the control of apical dominance, differentiation of the vascular tissues, branching, plant growth, morphogenesis, plant habit, determination and maintenance of tissue polarity, fruit ripening, and/or abscission.
9. DNA sequences according to one of the preceding claims derived from Arabidopsis thaliana.
10. DNA sequences according to one of the preceding claims that code for the pin1 gene product or an active fragment thereof.
11. DNA sequences according to one of the claims 1 to 9 that code for the pin2 gene product or an active fragment thereof.
12. SEQ:NO 11 (cDNA clone pcpin1/23 of the pin1 gene):

1 GTCTCGAGTT TTTTCTTCTT TTTTCCCTCT CACCACTTCT CTCTCAAAC  
 51 AAAGACAAAA GCTCTTCTCT CTTCCTCTC TCTTCTCCGG CGAACAAAA  
 101 GATGATTACG GCGGCGGACT TCTACCACGT TATGACGGCT ATGGTTCCGT  
 151 TATACGTAGC TATGATCCTC GCTTACGGCT CTGTCAAATG GTGGAAAATC  
 201 TTCACACCAG ACCAATGCTC CGGCATAAAC CGTTTCGTCG CTCTCTTCGC  
 251 CGTTCCTCTC CTCTCTTTCC ACTTCATCGC CGCTAACAAC CCTTACGCCA  
 301 TGAACCTCCG TTTCTCTGCC GCAGATTCTC TCCAGAAAGT CATTGTCTCT  
 351 TCTCTCTCT TCTCTGGTG CAACTCAGC CGCAACGGTT CTTTAGATTG  
 401 GACCATAACT CTCTTCTCTC TCTCGACACT CCCCAACACT CTAGTCATGG  
 451 GGATACCTCT TCTCAAAGGC ATGTATGGTA ATTTCTCCGG CGACCTCATG  
 501 GTTCAAATCG TTGTTCTTCA GTGTATCATT TGGTACATAC TCATGCTCTT  
 551 TCTCTTTGAG TACCGTGGAG CTAAGCTTTT GATCTCCGAG CAGTTTCCAG  
 601 ACACAGCAGG ATCTATTGTT TCGATTCTATG TTGATTCCGA CATTATGTCT  
 651 TTAGATGGAA GACAACCTTT GGAAACTGAA GCTGAGATTA AAGAAGATGG  
 701 GAAGCTTCAT GTTACTGTTC GTCGTTCTAA TGCTTCAAGG TCTGATATTT  
 751 ACTCGAGAAG GTCTCAAGGC TTATCTGCGA CACCTAGACC TTCGAATCTA  
 801 ACCAACGCTG AGATATATTC GCTTCAGAGT TCAAGAAACC CAACGCCACG  
 851 TGGCTCTAGT TTTAATCATA CTGATTTTTA CTCGATGATG GCTTCTGGTG  
 901 GTGGTCGGAA CTCTAACTTT GGTCTGGAG AAGCTGTGTT TGGTTCTAAA  
 951 GGTCTACTC CGAGACCTTC CAACTACGAA GAAGACGGTG GTCCTGCTAA  
 1001 ACCGACGGCT GCTGGAACCTG CTGCTGGAGC TGGGAGGTTT CATTATCAAT  
 1051 CTGGAGGAAAG TGGTGGCGGT GGAGGAGCGC ATTATCCGGC GCCGAACCCA  
 1101 GGGATGTTTT CGCCCAACAC TGGCGGTGGT GGAGGCACGG CGGCGAAAGG  
 1151 AAACGCTCCG GTGGTTGGTG GGAAAAGACA AGACGGAAAC GGAAGAGATC  
 1201 TTCACATGTT TGTGTGGAGC TCAAGTGCTT CGCCGGTCTC AGATGTGTTC  
 1251 GGCAGTGGAG GAGGAAACCA CCACGCCGAT TACTCCACCG CTACGAACGA  
 1301 TCATCAAAAG GACGTTAAGA TCTCTGTACC TCAGGGGAAT AGTAACGACA  
 1351 ACCAGTACGT GGAGAGGGAA GAGTTTAGTT TCGGTAACAA AGACGATGAT

1401 AGCAAAGTAT TGGCAACGGA CGGTGGGAAC AACATAAGCA ACAAACGAC  
 1451 GCAGGCTAAG GTGATGCCAC CAACAAGTGT GATGACAAGA CTCATTCTCA  
 5 1501 TTATGGTTTG GAGGAAACTT ATTCGTAATC CCAACTCTTA CTCCAGTTTA  
 1551 TTCGGCATCA CCTGGTCCCT CATTTCTTC AAGTGGAACA TTGAAATGCC  
 1601 AGCTCTTATA GCAAAGTCTA TCTCCATACT CTCAGATGCA GGTCTAGGCA  
 10 1651 TGGCTATGTT CAGTCTTGGG TTGTTTCATGG CGTTAAACCC AAGAATAATA  
 1701 GCTTGTGGAA ACAGAAGAGC AGCTTTTGCG GCGGCTATGA GATTTGTCGT  
 1751 TGGACCTGCC GTCATGCTCG TTGCTTCTTA TGCCGTTGGC CTCCGTGGCG  
 1801 TCCTCCTCCA TGTTGCCATT ATCCAGGCAG CTTTGCCGCA AGGAATAGTA  
 15 1851 CCGTTTGTGT TTGCCAAAGA GTATAATGTG CATCCTGACA TTCTTAGCAC  
 1901 TGCGGTGATA TTTGGGATGT TGATCGCGTT GCCCATAACT CTTCTCTACT  
 1951 ACATTCTCTT GGGTCTATGA AGAGATATTA CCAAACACA GGGACTTTGT  
 20 2001 TTTATTCTTT TGTGGGATGA TGAATTGTGA AAAGAACAAT GCCCTTTTTG  
 2051 TTGAAAACCC ACAAATTAAA TCAGAAGCAG CTTTAGAGAA TCTTTGAGGA  
 2101 TAATTGAAGC TCTTGAAGAA GAGAAGAAGA AGGAGACTTA AGTAGGAGCT  
 25 2151 CAGCAAGTTT TACCTTTTTT TTAATTTTAA TGAACATTCG TGTTCCTCT  
 2201 TTTGGTAGGT TTTAGGAATT TGTAAGCT TTGGCTACTT TTAGTGAATT  
 2251 AAAAACGTTA AGGAAAATAT CAAAAA AAAA AAAA AA

13. SEQ:NO 12 (cDNA clone pcpin2/4 of the pin2 gene):

1 GGCACGAGCC GGTTTCGTTGC GGTTCGCG GTTCCTCTTC TCTCTTTCCA  
 51 TTTCATCTCC TCCAATGATC CTTATGCAAT GAATTACCAC TTCCTCGCTG  
 101 CTGATTCTCT TCAGAAAGTC GTTATCCTCG CCGCACTCTT TCTTTGGCAG  
 40 151 GCGTTTAGCC GCAGAGGAAG CCTAGAATGG ATGATAACGC TCTTTTCACT  
 201 ATCAACACTG CCTAACACGT TGGTAATGGG AATCCCATTG CTTAGGGCGA  
 251 TGTACGGAGA CTTCTCCGGT AACCTAATGG TGCAGATCGT GGTGCTTCAG  
 301 AGCATCATAT GGTATACATT AATGCTCTTC TTGTTTGAGT TCCGTGGGGC  
 45 351 TAAGCTTCTC ATCTCCGAGC AGTTCGCGA GACGGCTGGT TCAATTACTT  
 401 CCTTCAGAGT TGACTCTGAT GTTATCTCTC TTAATGGCCG TGAACCCCTC  
 451 CAGACCGATG CGGAGATAGG AGACGACGGA AAGCTACACG TGGTGGTTTCG  
 50 501 AAGATCAAGT GCCGCCTCAT CAATGATCTC TTCATTCAAC AAATCTCACG  
 551 GCGGAGGACT TAACTCCTCC ATGATAACGC CGCGAGCTTC AAATCTCACC  
 601 GCGGTAGAGA TTTACTCCGT TCAATCGTCA CGAGAGCCGA CGCCGAGAGC  
 55 651 TTCTAGCTTT AATCAGACAA ATTTCTACGC AATGTTTAA GCAAGCAAAG  
 701 CTCCAAGCCC TCGTCACGGT TACTAATA GCTACGGCGG CGCTGGAGCT



751 GGTCCAGGTG GAGATGTTTA CTCACTTCAG TCTTCTAAAG GCGTGACGCC  
 801 GAGAACGTCA AATTTTGATG AGGAAGTTAT GAAGACGGCG AAGAAAGCAG  
 851 GAAGAGGAGG CAGAAGTATG AGTGGGGAAT TATACAACAA TAATAGTGTT  
 901 CCGTCGTACC CACCGCCGAA CCCAATGTTC ACGGGGTCAA CGAGTGGAGC  
 951 AAGTGGAGTC AAGAAAAAGG AAAGTGGTGG CGGAGGAAGC GGTGGCGGAG  
 1001 TAGGAGTAGG AGGACAAAAC AAGGAGATGA ACATGTTTCGT GTGGAGTTCG  
 1051 AGTGCTTCTC CGGTGTCGGA AGCCAACGCG AAGAATGCTA TGACCAGAGG  
 1101 TTCCTCCACC GATGTATCCA CCGACCCTAA AGTTTCTATT CCTCCTCAGC  
 1151 ACAACCTCGC TACTAAAGCG ATGCAGAATC TGATAGAGAA CATGTCACCG  
 1201 GGAAGAAAAG GGCATGTGGA AATGGACCAA GACGGTAATA ACGGGGGAAA  
 1251 GTCACCTTAC ATGGGCAAAA AAGGTAGCGA CGTGAAGAC GCGGTCCCG  
 1301 GTCCTAGGAA ACAGCAGATG CCGCCGGCGA GTGTGATGAC GAGACTAATT  
 1351 CTGATAATGG TTTGGAGAAA ACTCATTGCGA AACCCTAACA CTTACTCTAG  
 1401 TCTCTTTGGC CTTGCTTGGT CCCTTGCTC TATCAAGTGG AATATAAGA  
 1451 TGCCAACGAT AATGAGTGGA CCGATTTGCGA TATTATCTGA TGCTGGTCTT  
 1501 GGAATGGCTA TGTTTAGTCT TGGTCTATTT ATGGCATTGC AACCAAAGAT  
 1551 TATTGCGTGC GGAAAATCAG TAGCAGGGTT TGCATGGCC GTAAGGTTCT  
 1601 TGA CTGGACC AGCCGTGATC GCAGCCACCT CAATAGCAAT TGGTATTCGA  
 1651 GGTGATCTCC TCCATATCGC CATCGTTCAG GCTGCTCTTC CTCAAGGAAT  
 1701 CGTTCCTTTT GTTTTCGCCA AAGAATATAA CGTCCATCCT GATATTCTCA  
 1751 GCACTGCGGT TATATTCGGA ATGCTGGTTG CTTTGCCTGT AACAGTACTC  
 1801 TACTACGTTT TTTTGGGGCT TTAAGTTATT ATCAAAACGT ATTTGCAAAT  
 1851 AAAAGGCGAT ACGACCCAAA GGTGATTTTT TTTCAAACGA AAAAGAATAA  
 1901 TTACAAGAAC GAAAAAAGAC TAATTCCAGG TCAGAGGCTT AGGTGTATGG  
 1951 GACCATGCAA TGTCGCATTA ATTAAATTAT AGCATATGAT AGTCGAAAAT  
 2001 TTAGATAACT TTGTATAATT AATTATATGC ACATGCATGT ACGTGACTTT  
 2051 GTAGTTTTTG TTACATTTAT TAAATTTTTG GGATGTGCAA GTACAATTAT  
 2101 TACTAAAAAA AAAAAA

14. Proteins or fragments thereof encoded by the DNA sequences according to one of the preceding claims.

15. Proteins or fragments thereof encoded by the pin1 gene.

16. Proteins or fragments thereof encoded by the pin2 gene.

17. Protein encoded by the DNA sequence according to claim 12 or fragments thereof.

18. Protein encoded by the DNA sequence according to claim 13 or fragments thereof.

19. Proteins or fragments thereof according to one of claims 14-18 from Arabidopsis thaliana.

20. Nucleic acid molecules comprising the coding region of the pin1 gene, the pin2 gene or fragments thereof.
21. Nucleic acid molecules according to claim 20 wherein said coding region is combined with regulatory sequences.
- 5 22. Nucleic acid molecules according to claim 20 or 21 wherein said regulatory sequences control tissue-specific, developmental specific and/or constitutive gene expression.
23. Nucleic acid molecules according one of claims 20 to 22 wherein said regulatory sequences control inducible gene expression and the induction stimulus can be abiotic or biotic.
- 10 24. Nucleic acid molecules according to one of claims 20 to 23 comprising additional enhancer, transposon and/or ther regulatory sequences.
- 25 25. Nucleic acid molecules according to one of claims 20 to 24 wherein said coding region or fragments thereof are in the antisense orientation.
26. Vectors comprising a DNA sequence according to claims 1 to 13 or a nucleic acid molecule according to claims 20 to 25.
- 20 27. Microorganism or animal cells containing a DNA sequence according to one of the claims 1 to 13 or 20 to 26.
28. E. coli clone DSM 10712 (containing pcpin1/23).
- 25 29. Plants containing at least one of the DNA sequences according to one of claims 1 to 13, at least one of the nucleic acid molecules according to one of claims 20 to 26, or at least one of the microorganisms according to claims 27 or 28, as well as parts and products of these plants, including protoplasts, plant cells, calli, seeds, tubers, cuttings, etc., as well as the progenies of these plants.
- 30 30. Plants according to claim 29 wherein polar auxin transport or auxin activity is altered in said plants.
31. Plants according to claim 29 or 30 wherein plant growth, architecture, branching, morphogenesis, differentiation of the vascular tissues, abscission, flowering, fruit ripening, and/or other auxin controlled developmental processes are altered.
- 35 32. Dicotyledons according to one of claims 29 to 31.
33. Monocotyledons according to one of claims 29 to 31.
34. Plants according to claim 33 wherein said plant is wheat, maize, rye, oats, millet, barley, rice, maize.
- 40 35. Gymnosperms according to one of claims 29 to 31.
36. Plants according to claim 35 wherein said plant is poplar, picea or eucalyptus.
- 45 37. Method for producing plants, plant parts, plant products according to one of claims 29 to 36.
38. Use of DNA sequences and/or nucleic acid molecules according to one of claims 1 to 13 or 20 to 26 for the isolation, and use of fragments thereof or of the gene product or fragments thereof for the isolation of DNA sequences that code for proteins involved in polar auxin transport.
- 50 39. Use of the proteins according to one of claims 14 to 19 and the use of fragments thereof for the isolation of proteins involved in polar auxin transport.
40. Use according to claim 38 or 39 for the isolation of pin genes, fragments thereof or homologous DNA sequences or of pin proteins.
- 55 41. Use of DNA sequences and/or nucleic acid molecules according to one of claims 1 to 13 or 20 to 26 for producing plants which have an altered polar auxin transport or auxin activity.

**EP 0 814 161 A1**

42. Use according to claim 37 for producing plants in which plant growth, wood production, architecture, branching, morphogenesis, differentiation of the vascular tissues, abscission, flowering, fruit ripening, root formation and/or other auxin controlled developmental processes are altered.

5

10

15

20

25

30

35

40

45

50

55

Fig. 1

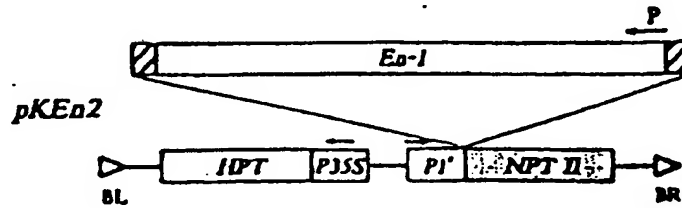


Fig. 2

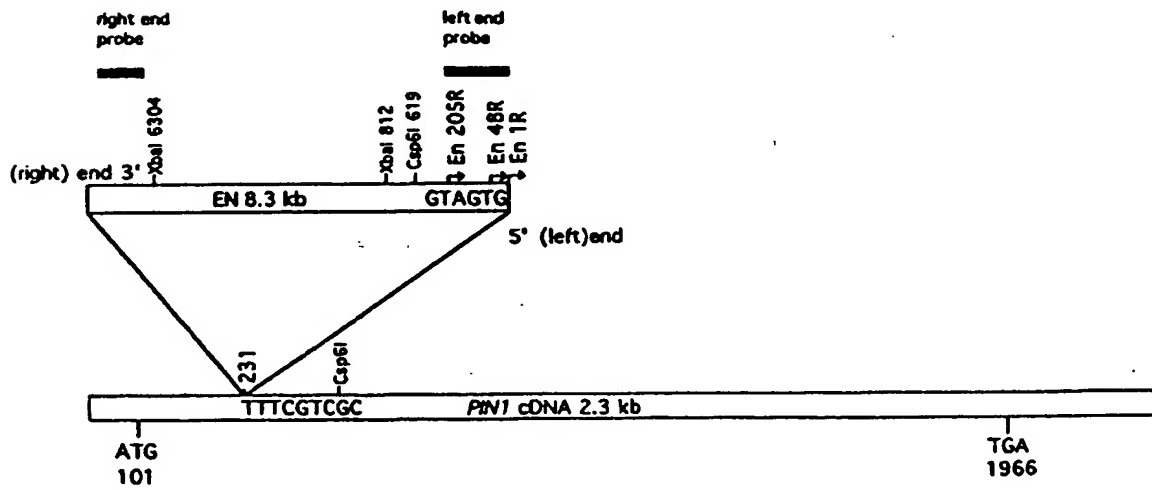


Fig. 3

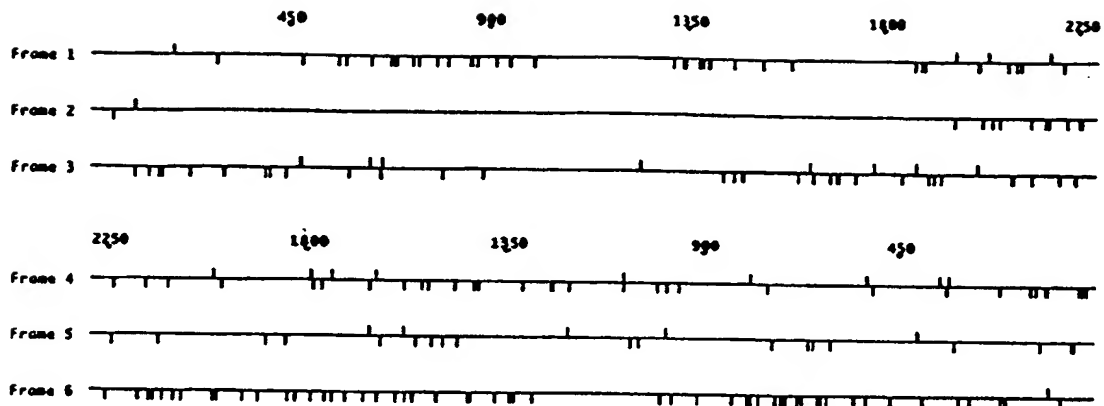


Fig. 4

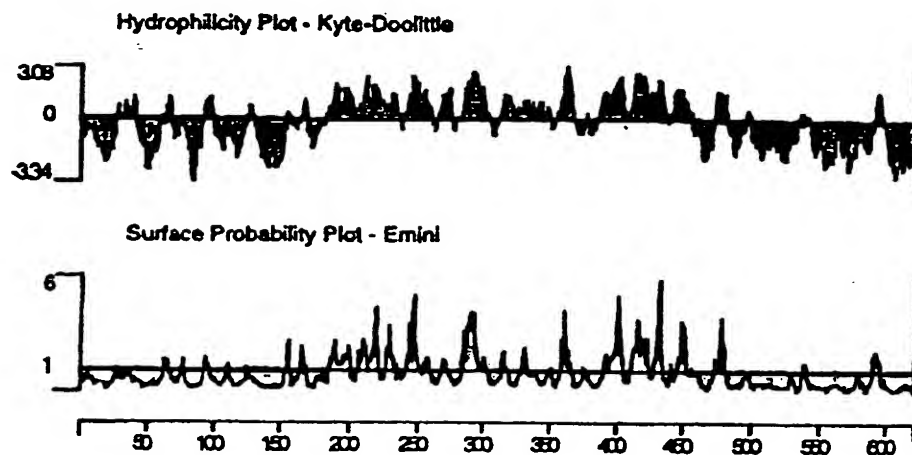


Fig. 5

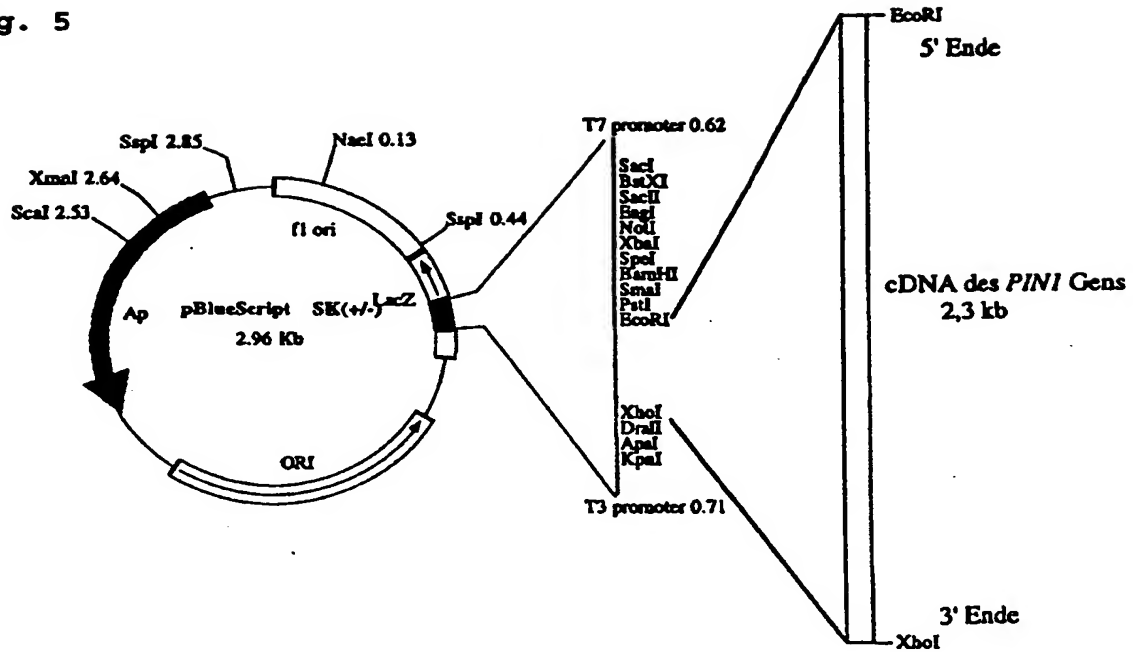
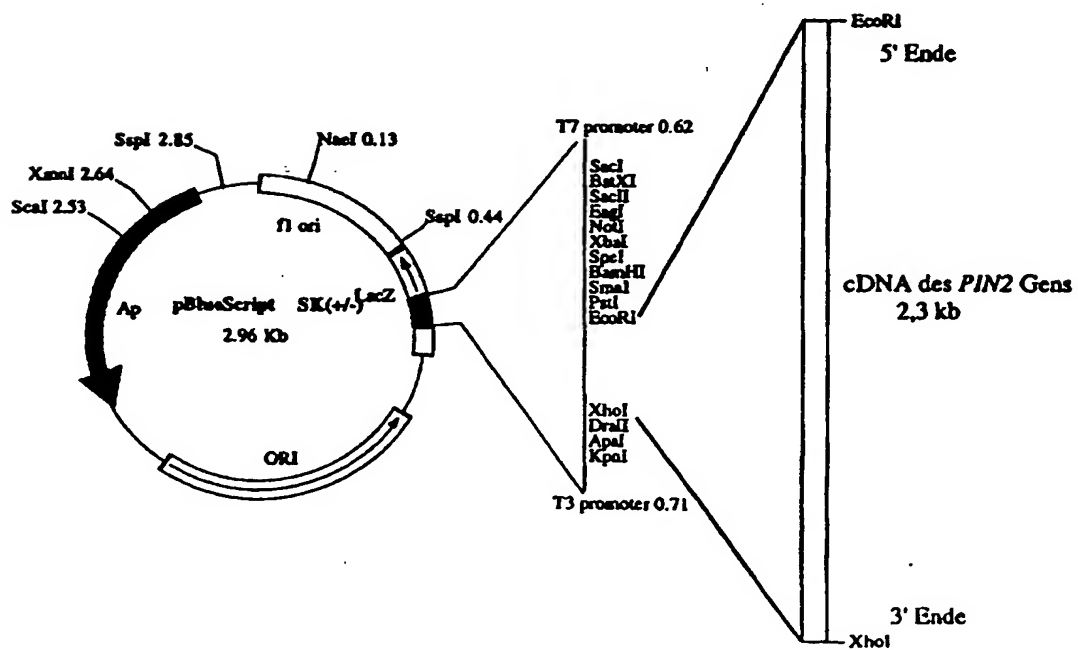


Fig. 6





European Patent  
Office

# EUROPEAN SEARCH REPORT

Application Number  
EP 96 11 0088

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	THE PLANT CELL, vol. 4, February 1992, pages 193-201, XP002024566 PALME, K., ET AL. : "MOLECULAR ANALYSIS OF AN AUXIN BINDING PROTEIN GENE LOCATED ON CHROMOSOME 4 OF ARABIDOPSIS"	1-5,14, 19,26, 27,38,39	C12N15/29 C12N15/82 C07K14/415 A01H5/00 C12Q1/68
Y	* the whole document *	29-34, 37,41,42	
	---		
X	THE PLANT JOURNAL, vol. 4, no. 3, 1993, pages 423-432, XP002024567 SCHWOB, E., ET AL. : "MOLECULAR ANALYSIS OF THREE MAIZE 22 kDa AUXIN-BINDING PROTEIN GENES - TRANSIENT PROMOTER EXPRESSION AND REGULATORY REGIONS"	1-5,14, 26,27	
Y	* the whole document *	29-34, 37,41,42	
	---		
X,D	THE PLANT JOURNAL, vol. 8, no. 4, 1995, pages 505-520, XP002024568 BENNETT, S.R.M., ET AL. : "MORPHOGENESIS IN PINOID MUTANTS OF ARABIDOPSIS THALIANA"	1-4,6-9	TECHNICAL FIELDS SEARCHED (Int.Cl.6)
Y	* the whole document *	29-34, 37,41,42	C07K C12N A01H C12Q
	---		
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 83, October 1986, pages 7277-7281, XP002024569 LEE, J.S.L., ET AL. : "MOLECULAR CHARACTERIZATION AND PHYLOGENETIC STUDIES OF A WOUND-INDUCIBLE PROTEINASE INHIBITOR I GENE IN LYCOPERSICON SPECIES"	1,2,14, 26,27	
Y	* the whole document *	29-34, 37,41,42	
	---		
	-/--		
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 5 February 1997	Examiner Holtorf, S
<p><b>CATEGORY OF CITED DOCUMENTS</b></p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons</p> <p>&amp; : member of the same patent family, corresponding document</p>			

EPO FORM 150 (01/92) (P0401)



European Patent  
Office

## EUROPEAN SEARCH REPORT

Application Number  
EP 96 11 0088

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	EMBL SEQUENCE DATA LIBRARY, HEIDELBERG , GERMANY, XP002024658 NEWMAN, T., ET AL . : "GENES GALORE: A SUMMARY OF METHODS FOR ACCESSING RESULTS FROM LARGE-SCALE PARTIAL SEQUENCING OF ANONYMOUS ARABIDOPSIS cDNA CLONES"	1,2, 9-13,20, 26,27	
Y	ACCESSION NO. T43636 ---	29-34, 37,41,42	
Y	WO 96 00291 A (RES CORP TECHNOLOGIES INC) 4 January 1996 * the whole document *	29-34, 37,41,42	
A,D	THE PLANT CELL, vol. 3, July 1991, pages 677-684, XP002024570 OKADA, K., ET AL . : "REQUIREMENT OF THE AUXIN POLAR TRANSPORT SYSTEM IN EARLY STAGES OF ARABIDOPSIS FLORAL BUD FORMATION" * the whole document *	1-42	
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 5 February 1997	Examiner Holtorf, S
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document	
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			

EPO FORM 1503 01.92 (P04C01)